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THE GOORMAGHTIGH CELLS IN THE NORMAL AND DISEASED HUMAN KIDNEY*

THEIR POSSIBLE RELATIONSHIP TO RENAL HYPERTENSION

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Recent experimental studies of hypertension in animals have focused attention anew on certain special structures present in the mammalian kidney.[1-6] These are the cell groups situated in the wall of the preglomerular arteriole, the juxtaglomerular apparatus of Goormaghtigh; and the specialized epithelial cells, the macula densa, present in the distal convoluted tubule. Indeed, the suggestion has been made that the juxtaglomerular apparatus may be concerned with the elaboration of a pressor substance.[7-14] During the past few years I have been studying these cell groups at the vascular pole of the glomerulus in the kidneys of human patients with and without hypertension, and wish to report certain findings which may have a bearing on this subject.

MATERIAL

Approximately 400 human kidneys were obtained either from the living patient or at autopsy. As soon as possible, usually within from ½ to 4 hours after removal, the tissues, cut into small blocks, were fixed in Zenker's or Bouin's solution. Sometimes Zenker's solution was injected into the renal artery while the organs were still *in situ*, to obtain more prompt fixation. Thin slices were dehydrated, cleared, embedded in paraffin and sections were cut serially at 4 to 6 μ. These were stained by the following methods: Goldner's modification of Masson's trichrome method, Mallory's aniline blue, Weigert's hematoxylin, Heidenhain's azan-carmine and Lillie's Biebrich-scarlet-red. Sometimes other stains were employed. Preparations stained by hematoxylin and eosin were also made and served as controls.

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Observations

In the human kidney, as in the kidneys of animals, a group of distinct cells is seen at the vascular pole of many but not all glomeruli. These cells are located between the afferent and efferent arterioles, frequently appearing to surround, usually eccentrically, the afferent vessel. Occasionally they extend a short way into the glomerulus along a capillary of the tuft. In this location they maintain a close relationship to the macula densa of the distal convoluted tubule from which no visible membrane seems to separate them. These cell structures are not confined to the vascular pole of the glomerulus. They are found most frequently in this situation but they also occur singly or in smaller groups along the interlobular artery and even the terminal artery. In the afferent arteriole they often form the entire vessel wall, being separated from the lumen by endothelial cells only. In the larger vessels they occupy the outer third of the media, often being dispersed irregularly among the smooth muscle cells, or are present only in the adventitia.

In recognition of the man who first studied and described these cells most extensively, I am calling them, as noted elsewhere,[13,14] "Goormaghtigh cells." In the human kidney they are large, polygonal and have indistinct cell outlines. Although they are usually closely packed, delicate argyrophilic fibrils, possibly of nervous origin, can often be detected between them. Their cytoplasm is scanty, usually appears vacuolated and sometimes contains a few fine fibrillar structures, although it is commonly afibrillar. Occasionally a few, fine fuchsinophilic granules, very difficult to differentiate from broken myofibrils, are present. The nucleus is small, round and vesicular, and usually situated in the center of the cell but sometimes found eccentrically. Frequently a clear halolike zone surrounds it.

Because of the fact that Goormaghtigh cells are so frequently situated near or among smooth muscle cells and occasionally contain broken myofibril-like structures in their cytoplasm, it has been suggested that they originate from muscle cells. Indeed, I was able to find what appeared to be actual transitional stages from one cell type to the other. I am therefore inclined to believe that they originate from smooth muscle cells.

The Goormaghtigh cells seem to be intrinsic parts of the human kidney, although their number may vary in individual cases. The youngest patient in whom I have observed them clearly was a boy of 3 months. They have also been found in the kidneys of a woman 78 years of age. No difference as to sex and race was found. They are more readily seen in the outer portions of the cortex, while in the deeper layers their appearance is less regular.

Since recent experimental work in dogs and rabbits by Goormaghtigh,[7-9] and in rabbits by Dunihue and Candon [11] and Dunihue,[12] seems to indicate that certain changes, such as hypertrophy and hyperplasia of these cells with concomitant alterations of the granular contents of the cytoplasm, may occur in experimental renal ischemia, an attempt was made to compare human kidneys which were definitely pathological with apparently "normal" kidneys. A large number of cases of so-called benign progressive arteriolar nephrosclerosis, three cases of malignant nephrosclerosis and three cases of pyelonephritis with secondary vascular changes, all with clinical hypertension, were examined.

It was found that there were definite hypertrophy and hyperplasia of Goormaghtigh cells in the kidneys of patients with chronic pyelonephritis with vascular changes, moderately advanced and advanced arteriolar nephrosclerosis and one case of malignant nephrosclerosis, all with clinical hypertension. However, in contrast to the experimental animal, there were no detectable associated alterations in their cytoplasm. Granules were present in scanty amount but they were neither more definite nor distinct than they were in the Goormaghtigh cells of normal kidneys.

In addition, in those cases where such severe vascular lesions were present that the cells which normally form the vessel wall underwent atrophic and degenerative changes, the Goormaghtigh cells remained remarkably well preserved. Moreover, in many of the interlobular arteries whose walls were markedly sclerosed and even hyalinized, groups of well preserved Goormaghtigh cells were frequently observed in or around the damaged media.

Comment

From these observations, together with those made by others, it now appears justifiable to add to the known structures of the normal human nephron two others which are regularly found in the renal cortex. These are the Goormaghtigh cells of the arteriolar walls and the macula densa of the distal convoluted tubule.

The nature of the Goormaghtigh cells is uncertain. Ruyter,[1] Oberling,[2] Goormaghtigh [3] and Zimmermann [4] noted that they were usually afibrillar and occurred constantly in the media of the arterioles. They considered them to be specially differentiated leiomyoblasts. Although in general they were afibrillar, Ruyter and Goormaghtigh found some cells which contained a few myofibrils, for which Goormaghtigh later coined the adjective "paucifibrillar." Ruyter, studying these cells in the mouse, was the first to demonstrate mitochondrial granules in their protoplasm, while Goormaghtigh [3] somewhat later found granules in

the afibrillar cells of the renal arterioles of the cat. Some investigators, notably Ruyter [1] and Schumacher,[15] thought that the afibrillar cells were epithelioid in character, but today it is generally considered that they are actually transformed smooth muscle cells. I am inclined to agree with this interpretation, although often it was extremely difficult to differentiate between broken-up myofibrils and separate acidophilic granules in paucifibrillar cells. Indeed, there seems to be good evidence for the transition of the smooth muscle cells into paucifibrillar and afibrillar cells. Their location in the arteriolar wall as well as their cytological characteristics favor such a transition.

Although Goormaghtigh cells were actually found some distance away from the glomerulus—in the interlobular artery and the terminal artery, for example—it would appear that their usual location at the vascular pole and their close proximity to the macula densa must be of significance. Thus it could readily be assumed that these cell groups and the macula densa might form a unit which had a special physiological function to perform.

In his early work Goormaghtigh [3] thought that the physiological significance of the afibrillar cells was identical with that of the epithelioid cells described in arteriovenous anastomoses occurring in other parts of the body (Schumacher,[15] Vastarini-Cresi,[16] Grosser,[17] Masson,[18-20] Clara,[21] Grant [22] and Popoff [23]). He reasoned that the afibrillar leiomyoblasts of the renal arterioles, situated as they are between a contractile arteriolar segment and a capillary network, could bring about the temporary exclusion of the glomerular loops from the renal circulation. This, of course, would make possible diversion of the blood through aglomerular arteriovenous shunts (Oliver and Luey,[24] Dehoff [25] and Spanner [26]). Moreover, the observation of an intricate sympathetic nerve plexus among these cells led Goormaghtigh to believe that in them could originate a reflex which controlled the contraction or relaxation of the smooth muscle cells of the media. The smooth muscle cells would thus control the blood supply of the glomerulus.

Clara,[27] on the other hand, thought that turgescence of these "epithelioid" cells could bring about closure of the afferent arteriole while their relaxation would open the vessel again into the glomerulus. He believed that the physiochemical condition of the glomerular filtrate in the distal convoluted tubule provided the stimulus for the closing and opening mechanism via the macula densa, which acted as a "receiving" unit.

The intimate relationship of Goormaghtigh cells and macula densa at the vascular pole of the glomerulus in normal human kidneys could support either one of these hypotheses. Moreover, either hypothesis could be reconciled with much of the recent experimental work in renal

physiology and function (Okkels and Péterfi [28] and Okkels [29]). However, my observations in pathological kidneys as well as experimental work by others [7-9, 11, 12] point to the possibility of a third hypothesis which is incompatible with both of the foregoing.

This hypothesis was first propounded by Goormaghtigh [7] as a result of certain studies made in 1939. In the course of experiments on renal hypertension in cats and rabbits, he drew attention to what he called the endocrine character of the afibrillar cells. This was a totally new concept. In four rabbits, in particular, he observed a variable number of secretory granules in the cells of the juxtaglomerular apparatus. At first these cells were afibrillar; then they became loaded with acidophilic granules which finally turned toward basophilia. From these observations he concluded that:

"L'ischémie rénale provoque l'hypertrophie et la multiplication de certaines cellules de la média des artérioles du rein qui normalement sont dépourvues de myofibrilles et possèdent chez le lapin des caractères cytologiques de cellules endocrines. A condition d'être assez accentuée, elle provoque la transformation des cellules musculaires à myofibrilles en cellules afibrillaires qui deviennent toujours granuleuses chez le lapin et parfois aussi d'une manière plus discrète chez le chien. Quand l'ischémie dure plusieurs mois, de nombreux éléments afibrillaires des artérioles rénales du chien deviennent granuleux et acquièrent des caractères cytologiques endocrines comme chez le lapin." [Experimental renal ischemia produces hypertrophy and hyperplasia of certain cells of the media of the renal arterioles which normally do not have myofibrils and which in the rabbit possess cytological characteristics of endocrine cells. If the ischemia is well marked, it produces the transformation of the myofibrillar smooth muscle cells into afibrillar cells which always become granular in the rabbit and sometimes to a slighter degree in the dog. When the ischemia lasts for several months, numerous afibrillar elements of the renal arterioles of the dog become granular and take up endocrine cytological characteristics, as seen in the rabbit.]

It should be noted, however, according to Goormaghtigh,[7] that in the rabbit these granular changes are found chiefly in the afibrillar cells of the superficial glomeruli, while deeper in the cortex the granularity is much less pronounced.

This hypothesis, based as it is on morphological changes alone, becomes important only in the light of physiological experiments on renal hypertension. The earliest investigations in the latter field were those of Tigerstedt and Bergman,[30] who, in 1898, isolated from the cortex of various mammalian kidneys a hypertensive substance which they called "renin." Nearly 40 years later, Prinzmetal and Friedman,[31] working on dogs and cats, and Harrison, Blalock and Mason,[32] working on dogs, confirmed the work of Tigerstedt and Bergman. Many others [33-46] have done the same and isolated other additional substances, of which the importance in hypertension is today generally recognized. Although there is as yet no agreement as to the name or even the identity of the

renal hypertensive substance or substances, there is sufficient experimental evidence for their existence in the renal cortex and in the cortex alone. Since renin is found only in the renal cortex and since it is in this part of the kidney that the afibrillar cells become granular and show "endocrinic characteristics," Goormaghtigh suggested that these cells may be concerned with the production of the pressor substance.

As far as animal experiments are concerned, Dunihue and Candon,[11] and Dunihue,[12] who have produced hypertension by using Drury's [47] method and Page's [48] cellophane method, have recently been able to confirm Goormaghtigh's latest observations in the rabbit.

I am indebted to Dr. Dunihue for permitting me to examine some of his sections. They show beautifully, in the afibrillar cells, the granular changes which are found in association with experimental hypertension. However, in the human kidney the demonstration of intracellular granules is much more difficult than in the experimental animal. It is absolutely essential for the demonstration of these intracellular granules to obtain very fresh material, which, in the human, is not always possible. From my own observations in human kidneys as well as from those of others in the kidney of the dog, it seems that species differences exist in the Goormaghtigh cells which have now been described in most vertebrates.[49] It has already been mentioned that in the cases of hypertension with renal arteriolar damage which I studied there was uniform hypertrophy and hyperplasia of the Goormaghtigh cells, particularly in the afferent arterioles, but that the granular changes described in the experimental animal were not found. In addition, analogous to the findings of Goormaghtigh and Handovsky,[50] who fed dogs large doses of vitamin D_2 (calciferol) and noted that the afibrillar cells remained undamaged long after severe sclerosis and necrosis of the renal arteriolar wall had set in, in the kidneys of hypertensive patients well preserved Goormaghtigh cells were found in severely damaged arterioles.

These findings are significant in the light of the experiments which demonstrated the presence of a pressor substance in the cortex of the normal kidney. In experimental renal hypertension this pressor substance is increased and appears in the venous blood of the kidney. This suggests strongly that the hypertrophied and hyperplastic Goormaghtigh cells of the afferent arterioles of the glomerulus might be its source. Moreover, it is generally assumed that the vasoconstrictor effect of the pressor substance acts directly on the smooth muscle cells of the arteriolar media. Since it is known that this constriction is particularly pronounced in the efferent arterioles, does this suggest that the proximity of this vessel to the afferent arteriole, in which hypertrophy and

hyperplasia of Goormaghtigh cells is so pronounced, may be of any significance? Further work is certainly necessary to correlate these experimental findings with the observations made in the kidneys of hypertensive patients. In these organs the Goormaghtigh cells lack the morphological characteristics of endocrine function as defined by Goormaghtigh.

Thus in the absence of definitely demonstrable cytoplasmic granules in the Goormaghtigh cells of kidneys from patients with hypertension, the hypertrophy and hyperplasia of these cells appear at present to be the only evidence suggestive of an endocrine function which could be related to the production of a renal pressor substance.

Summary

Four hundred kidneys, removed either at operation or at autopsy, were examined and Goormaghtigh cells were regularly found at the vascular pole of those glomeruli which were situated in the outer part of the cortex. They appeared to be intrinsic parts of the human kidneys. They were situated close to the macula densa of the distal convoluted tubules. They also occurred in and along the wall of the afferent arteriole, the efferent arteriole, the interlobular artery and the terminal artery. Their location and their cytological characteristics would indicate that they are probably transformed smooth muscle cells of the vascular media. Although their close relationship to the macula densa suggests that they provide an opening and closing mechanism for the access of the blood to the glomerular tuft, my findings as well as recent work on experimental hypertension of renal origin cast considerable doubt on this theory and strongly suggest the possible endocrine character of the Goormaghtigh cells.

Note: I wish to express my gratitude to Dr. Arthur W. Wright, Professor of Pathology and Bacteriology, Albany Medical College, for his valuable assistance in carrying out this work.

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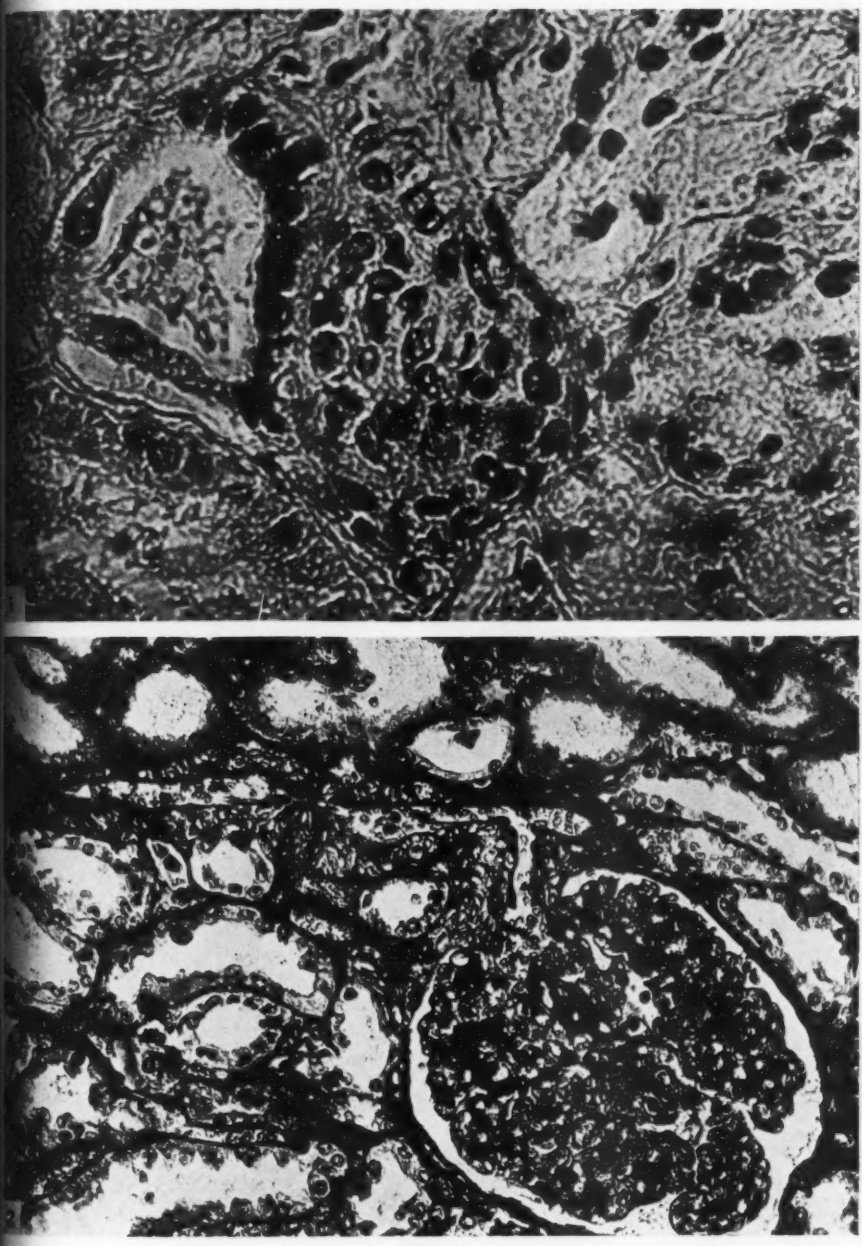
DESCRIPTION OF PLATES

PLATE 114

FIG. 1. Vascular pole of a glomerulus with juxtaglomerular apparatus and adjacent macula densa of a distal convoluted tubule. The Goormaghtigh cells of the juxtaglomerular apparatus and the tall epithelial cells of the macula densa are in close contact. The kidney was from a negro, 25 years old, who died of tuberculous meningitis. There was no renal pathology. Hematoxylin and eosin stain. × 545.

FIG. 2. Glomerulus, afferent arteriole and interlobular artery from a kidney of a white man, 40 years old, with moderate hypertension for several years. There is eccentric thickening of the arteriolar wall, particularly at the vascular pole of the glomerulus, and a moderate degree of hyalinization of the capillaries of the glomerular tuft. Mallory's aniline blue and Weigert's iron hematoxylin stain. × 275.

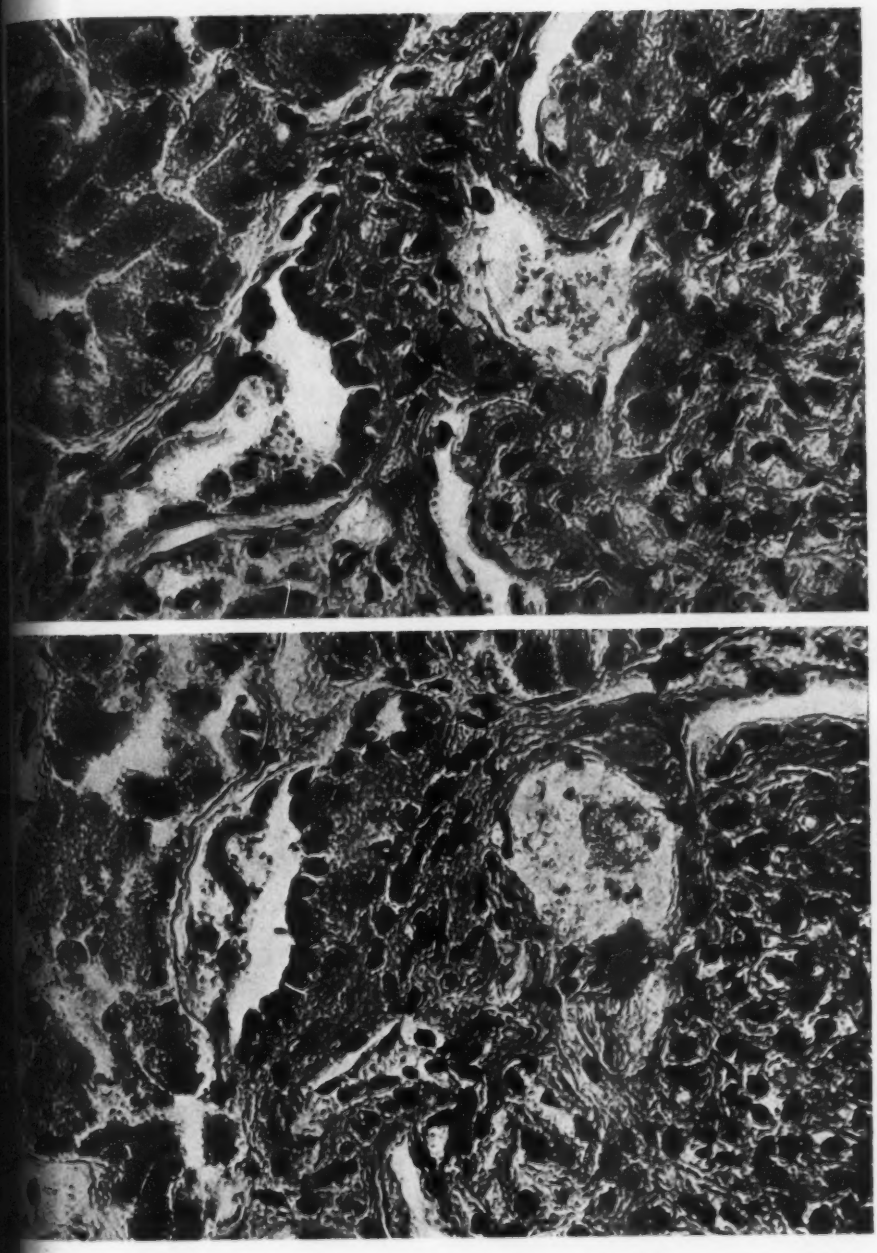


KAUFMANN Goormaghtigh Cells in the Human Kidney

PLATE 115

FIG. 3. Beginning sclerosis in the wall of an afferent arteriole with moderate hypertrophy and hyperplasia of Goormaghtigh cells. This was from the kidney of a white woman, 78 years old, with long-standing moderate hypertension. A well outlined macula densa of a distal convoluted tubule may be seen close to the afferent arteriole. Goldner's modification of Masson's trichrome stain. × 570.

FIG. 4. Another glomerulus, Goormaghtigh cells and macula densa, from the same patient as Figure 3. There is progressing sclerosis of the afferent arteriole and beginning disintegration of Goormaghtigh cells. Goldner's modification of Masson's trichrome stain. × 570.

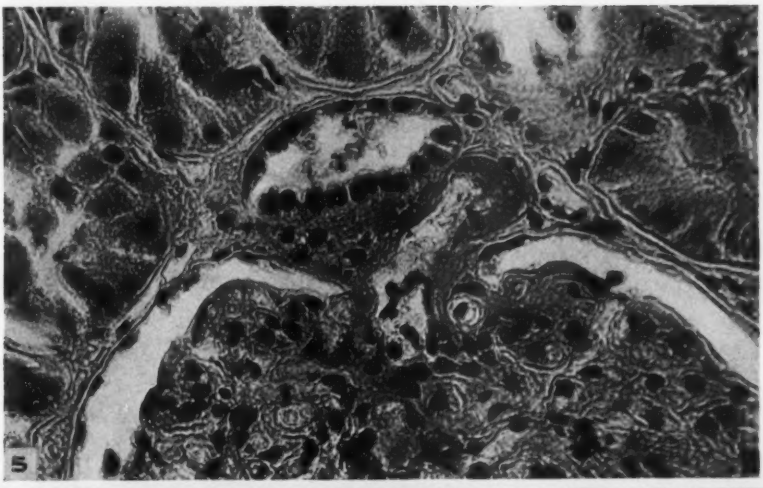


Hofmann Goormaghtigh Cells in the Human Kidney

PLATE 116

FIG. 5. A third glomerulus, Goormaghtigh cells and macula densa from the same patient as Figures 3 and 4. There is marked hyalinization of the wall of the afferent arteriole and almost complete disappearance of Goormaghtigh cells. Goldner's modification of Masson's trichrome stain. × 505.

FIG. 6. Interlobular artery from a kidney of a white man, 54 years old, with severe hypertension of several years' duration. (Blood pressure was 254/158 mm. of Hg on admission.) There is extreme sclerosis of the arterial wall with hypertrophied, hyperplastic and well preserved Goormaghtigh cells. Lillie's Biebrich-scarlet-red stain. × 505.



Kaufmann Goormaghtigh Cells in the Human Kidney

INTRANUCLEAR INCLUSIONS IN INFANCY*

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The question of the etiology and significance of inclusion bodies in the organs of infants has given rise to considerable speculation since Jesionek and Kiolemenoglou [1] first described them in 1904, in the lungs, kidneys and liver of an 8-month luetic fetus. In the same year, Ribbert [2] described similar structures in the parotid glands of two nonluetic infants and reported that he had previously (1881) observed them in a kidney of a luetic stillborn infant. In both reports it was concluded that these intranuclear inclusions most likely represented some one of the protozoa. Löwenstein,[3] in 1907, found intranuclear inclusions in both parotid glands in 4 of 30 infants and, in addition, described cytoplasmic inclusions. Pisanò,[4] in 1910, and Mouchet,[5] in 1911, also reported finding intranuclear inclusions in luetic infants. In the United States in 1910 and 1914, Smith and Weidman [6, 7] of Philadelphia described similar inclusions. These authors concluded that they were due to a protozoan to which they gave the name *Endamoeba mortinatalium*. No other important observations were made until 1920, when Jackson [8] described intranuclear inclusions in the salivary glands of guinea pigs, but these, too, were thought to be protozoa.

The protozoan theory was not discarded until 1921 when Goodpasture and Talbot [9] showed these inclusions to be products of modified cells, and compared them with the nuclear changes in the cutaneous lesions which Tyzzer [10] had described in varicella in 1906. It was in the same year (1921) that Lipschütz [11] reported the finding of intranuclear inclusions in the lesions of herpes in rabbits and man, and concluded that they represented specific reactions to a specific virus. However, in the following year, 1922, Müller [12] reported 3 cases of inclusions in the kidneys of infants and postulated that the "protozoan-like cells" represented degenerated tissue cells. In 1925, Von Glahn and Pappenheimer,[13] for the first time, reported the finding of similar inclusions in an adult. They described these lesions in the intestines, liver and lungs of a male, 36 years old, who died from ulcerative colitis, liver abscess and pneumonia. Walz,[14] in 1926, reported the case of a newborn infant with inclusions in the lungs, thyroid, liver, pancreas and kidneys, while Wagner,[15] in 1930, found inclusions in the sublingual gland, lungs, thyroid, liver, pancreas, kidneys and epididymis of a 2-week premature infant and inclusions in the parotid glands of 4

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infants. In 1932, Farber and Wolbach [16] reported by far the largest single series—22 cases in which the inclusions were found in the submaxillary glands alone, together with 2 cases in which inclusions were present in the viscera. In this series the submaxillary glands were studied in a series of 183 postmortem examinations on infants and the incidence of the inclusions was 12 per cent. These investigators described cytoplasmic inclusions which were frequently found in conjunction with the intranuclear inclusions.

Subsequently Rich [17] and also McCordock [18] and McCordock and Smith [19] have reported the finding of intranuclear inclusions associated with pertussis, and Goodpasture, Auerbach, Swanson and Cotter [20] have recently described 5 cases of virus pneumonia in infants in which intranuclear inclusions were present. These findings will be discussed in detail in a later paragraph.

In 1940, Vidari [21] reported a case with "protozoanlike cells" in the lungs, thyroid, liver and kidneys, associated with icterus neonatorum and erythroblastosis, and stated there were 5 similar cases in the literature.

Part 1

The patient, a male child of 3 years, was taken to the hospital because of convulsions and colonic spasms.

Little was known concerning the past history except that there had been no recent acute illnesses. Several siblings were living and well. On July 1, 1933, the patient fell from a coaster wagon, receiving a severe blow on the lumbar region, but except for a bruise there was no evidence of injury. Two days later the child fell from a porch and cried for a considerable period of time but moved about normally. On July 9, 1933, he suffered from what was termed a "convulsion," trembling and crying for short intervals. Following this convulsion, the patient was examined by a physician who was unable to find any evidence of disease. Three days later more convulsions took place and the child was hospitalized.

Physical examination at the time of admission showed a well nourished boy, lying on his back in bed, in a semistuporous condition. The temperature was normal. He could be roused by speech and by shaking but was apparently disoriented. Examination of the lungs, heart and abdomen presented no abnormalities. The pupils reacted to light. The tongue protruded in the midline without apparent tremor. The lips twitched constantly but were not drawn to either side. The right abdominal and the cremasteric reflexes were absent. The knee jerks could be elicited but were sluggish. The Kernig and Babinski reflexes were negative.

The convulsions became steadily more severe during the patient's 2 days in the hospital. He was incontinent, comatose and unable to retain food or fluid. On the second hospital day, marked swelling appeared over the left frontal region which, in a very short time, involved the lids of the left eye to such a degree that they could not be opened. The temperature remained normal until shortly before death when it rose sharply to 105° F. In addition, the patient became rigid for a short time preceding death.

A lumbar puncture was performed. The spinal fluid was not under increased pressure. Eight cc. of blood-tinged fluid was removed. Cultures of this fluid were negative.

Postmortem Examination

Autopsy was performed, but no gross abnormalities were found in any of the organs.

Sections were taken for study from the cerebrum, medulla, pons and cervical cord. These were fixed in Zenker's fluid and stained with phloxine and methylene blue. The inflammatory process present was widespread in all of the sections studied with the exception of those taken from the cord. Throughout the cortex and subcortical white matter of the cerebrum were focal areas infiltrated by polymorphonuclear leukocytic cells together with moderate numbers of mononuclear cells (Fig. 1). In the white matter these areas were distinctly perivascular while those in the gray matter were more diffuse. In a few of these foci in the gray matter the tissue was partially necrotic, but in other similar foci in both the gray and white matter there was no obvious necrosis. In some of these areas intranuclear inclusions could be seen in the nerve cells. The walls of many of the blood vessels of the parenchyma were necrotic and were infiltrated by polymorphonuclear leukocytes and macrophages (Fig. 2). There was considerable perivascular cuffing by lymphocytes. One intranuclear inclusion was found in a cell in a vessel wall.

Focal lesions similar to those described were found throughout the medulla. However, these lesions appeared less acute as there were larger numbers of mononuclear cells and fewer polymorphonuclear cells. These lesions were especially prominent in the vagal nuclei and intranuclear inclusions were quite numerous there.

The intranuclear inclusions were most prominent in nerve cells adjacent to the lesions described (Figs. 3 and 4). In cells in which the inclusions were well developed, the nuclear chromatin was marginated, leaving a clear zone between the nuclear membrane and the inclusion. The inclusions were definitely acidophilic and were quite granular in appearance. These inclusions did not resemble in any way those which are described as occurring in the nuclei of ganglion cells in poliomyelitis and other virus diseases.

At the time of autopsy, the nature of these lesions was not suspected and so no effort was made to recover a virus. However, the morphology of the lesions and particularly of the inclusion bodies strongly suggests a herpeslike virus as the etiological agent in this case. The negative bacterial cultures of the spinal fluid support this viewpoint. The vascular lesions deserve emphasis, as these may account for the large areas of necrosis. This is the fourth case to be reported, as far as can be determined, in which intranuclear inclusions similar to those of herpes have been found in the brain. Two cases were reported by

Dawson,[22, 23] one in 1933 and the second in 1934; and the third case was reported by Smith, Lennette and Reames[24] in 1941. This latter group of investigators, in addition to demonstrating the intranuclear inclusions, were able to recover and identify the virus of herpes simplex.

Part II

The patient, a female infant, 14 days old, was born at the Boston City Hospital on October 24, 1939. The mother, a 19-year-old primipara, was followed throughout her pregnancy in the out-patient department and appeared to be in excellent health. The pregnancy was uneventful. The Hinton test was negative. She had suffered from measles and chickenpox as a child; otherwise the past history was not remarkable.

Labor was at term and was uneventful. The infant weighed 2355 gm. (4 lbs. 14½ oz.) at birth. When examined shortly following birth, the infant appeared to be normal in all respects except for its small size. Because of this the patient was placed in an incubator and also given oxygen for the first day. She was fed 10 oz. of breast milk per day. The patient did well until the 11th day when she began to pass many stools. On the following day, she began to vomit and to refuse food. Jaundice was noticed and occult blood was found in the stools. She lost ground rapidly, becoming dehydrated and moribund despite supportive treatment, and died in her 14th day of life.

Postmortem Examination

Postmortem examination was performed 4½ hours following death. The body was that of a poorly nourished female infant weighing 1780 gm. There was a marked icteric tint in the skin and sclerae. With the exception of the brain, the organs showed no gross abnormalities. The leptomeninges were not remarkable and the convolutions were not flattened. There were irregular areas of softening in the right putamen and in the left basal ganglia, whose diameter varied from 1 to 1.5 cm. These areas were bright yellow. Scattered throughout these areas of softening were smaller pearly white, plaquelike masses which appeared to be slightly firmer than the uninvolved parenchyma and which measured from 1 to 3 mm. in diameter. Lesions similar to the above were found throughout the occipital lobe to such a degree as to involve approximately one-third of the lobe. There were no gross abnormalities of the vascular system of the brain.

On microscopical examination there was no evidence of disease except the intranuclear inclusions in the lungs, liver, pancreas and kidneys and the lesions in the brain. These intranuclear inclusions, except for those in the brain, were always found in epithelial-lined spaces, a finding which was first emphasized by Farber and Wolbach.[16] They usually appeared as oval, dense, homogeneous acidophilic bodies located in the center of the nucleus. They were usually separated from the nuclear membrane by a halo or clear zone, although occasionally the zone would be obliterated by an especially large inclusion. In the clear zone,

one or several dense, granular, round, basophilic masses suggesting chromatin could usually be found. In many cases there was beading of the nuclear membrane by dense, irregular, granular, basophilic material which was interpreted as being remnants of nuclear material (Fig. 5).

In many of the cells containing intranuclear inclusions, cytoplasmic inclusions were found as well. These were especially prominent in the kidney and pancreas. They appeared as irregular, finely granular, basophilic masses lying between the cell and nuclear membranes.

Occasionally, a few lymphocytes were found lying near the cells containing inclusions; otherwise, there was no apparent reaction in the surrounding tissue.

Microscopically, the areas of softening in the brain were found to be areas of marked necrosis with mononuclear cell infiltration. One intranuclear inclusion similar to those found in the other organs was found in the brain but it was not in or near an area of necrosis (Fig. 6). This finding is of considerable interest, as it is the first time such inclusions have been described as occurring in the brain.

This case is presented as an example of the so-called "inclusion disease" of infants; cases in which the inclusions are so widespread in the body are distinctly rare. Only 25 cases have been reported in which inclusions have been found in organs other than the parotid gland and in only 11 have they been found in several of the viscera, as in the present case. In the remaining 14 cases the inclusions were found only in single organs and in this group they were present most frequently in the lungs, kidneys, or liver.

These inclusions are morphologically indistinguishable from those found in the salivary glands of children and from those in the submaxillary glands of guinea pigs.[25, 26] However, while the disease of the submaxillary gland in guinea pigs has been shown to be due to a transmissible virus, no such results have been obtained in children.

Part III

Intranuclear inclusions were found in the lungs in four, and in the salivary glands in two autopsies of infants suffering from clinical whooping cough. All cases presented morphological evidence of pertussis.[27] Four of these infants died in from 2½ to 5 weeks following the onset of symptoms. No history could be obtained regarding the other two patients. It was felt that the period of time which elapsed from the onset to the time of death could be of little significance as an indicator of the presence of inclusions, as 31 cases were examined which fell into the same range of time without finding evidence of

inclusions. No other factor could be found which would give any indication of the presence of inclusions.

The inclusions found in the lungs were found in the cells lining the alveoli and bronchi and occasionally in the mucous glands of the bronchi and trachea. The cells containing the inclusions were approximately twice the size of the uninvolved cells and their cytoplasm was more basophilic. The inclusions themselves were acidophilic and were separated from the nuclear membrane by a clear zone. The nuclear membrane was often beaded by small fragments of chromatin. Large numbers of these cells lay free in the alveolar spaces. It was felt that these had become detached from the alveolar walls. The nuclei of a few of these cells contained two, or even three, inclusions. No cytoplasmic inclusions were found in the lung (Fig. 7).

The intranuclear inclusions found in the salivary glands (Fig. 8) were similar to those found in the lungs. In addition, cytoplasmic inclusions were frequently present in these cells. These were basophilic, granular, spherical to elliptical bodies lying between the nuclear and cell membranes. Salivary glands were not examined in any of the 4 cases in which inclusions were found in the lung or in the larger group of cases of pertussis, described below, in which no inclusions were found. As a matter of interest, salivary glands from 25 infants were studied but no inclusions were present. The lungs from 31 cases of pertussis were studied without finding inclusions of any type. This represents an incidence of 16 per cent of intranuclear inclusions in the lungs or parotid material studied, and an incidence of 11 per cent in the lungs alone.

Feyrter,[28] in 1927, reported 1 case of intranuclear inclusions in the lungs of an infant dying of pertussis, while Rich,[17] in 1932, reported 5 cases. McCordock and Smith,[19] in 1934, reported the finding of these inclusions in the lungs in 18 of 40 autopsies; that is, in 45 per cent of children dying from pertussis. The finding of intranuclear inclusions in association with pertussis has prompted the suggestion that the etiological agent of pertussis is a virus. There are other reasons aside from the finding of inclusions to suspect that a virus may play some part in the pathogenesis of whooping cough. A reason frequently advanced is that the interstitial bronchopneumonia, which may accompany whooping cough, is similar to that seen complicating measles and epidemic influenza and to the pneumonia produced experimentally in animals by intratracheal injection of various types of virus. Less importance must be attached to this since Sprunt, Martin and Williams [29, 30] and Cullota, Marting and Liebow [31, 32] were able to produce interstitial pneumonia by injection of pure cultures of Bordet-Gengou bacilli in rabbits, mice and ferrets.

Other arguments in favor of the virus theory are that whooping cough may be complicated by an encephalitis, as in the case of measles and influenza, and that patients recovering from an attack of whooping cough acquire a lasting immunity from the disease. Also, the highly contagious nature of pertussis is stressed. Rich, Long, Brown, Bliss and Holt [33] were able to produce in apes an upper respiratory infection, closely resembling the catarrhal stage of whooping cough, by the inoculation of bacteriologically sterile blood and filtered respiratory secretions obtained from early cases of human pertussis. In addition, they were able to transfer the agent from one ape to another by means of a bacteriologically sterile filtrate of the nasopharyngeal secretions. However, in other groups of apes they produced a condition simulating human pertussis in all respects by oral inoculation of pure cultures of Bordet-Gengou bacilli. Also, Macdonald and Macdonald [34] produced whooping cough by the instillation of pure culture of Bordet-Gengou bacilli into the noses and throats of four boys. A previous attempt to produce whooping cough in these children by the instillation of a culture filtrate was unsuccessful. Gallavan and Goodpasture [35] were able to reproduce the exact pathological lesions of human pertussis in the respiratory tract and lungs of the chick embryo by the infection of the chorioallantoic membrane and by the inoculation of the amniotic fluid with the Bordet-Gengou bacillus. They believed this to be conclusive proof that the pathogenic agent in whooping cough is the Bordet-Gengou bacillus. They did not consider the inclusion bodies to be of significance in pertussis because they are absent in the majority of cases and because they are found in infants not suffering from this disease. The suggestion has been made by Rich that the inclusions are the result of an initial virus infection which paves the way for the Bordet-Gengou bacillus. Rich also suggested the possibility of these inclusions resulting from aspirated herpes virus. It is evident that the etiology of these inclusions as well as their exact rôle in the pathogenesis of whooping cough is unknown.

Summary

1. Intranuclear inclusions similar to those of herpes are described in the brain of a child of 3 years.

2. A case of so-called "inclusion disease" of infants is reported.

3. Six cases are discussed in which intranuclear inclusions were found in the lungs or salivary glands of infants dying of pertussis.

Note: I wish to thank Dr. Leroy U. Gardner of the Saranac Laboratory for his kindness in supplying me the material for Part I.

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DESCRIPTION OF PLATES

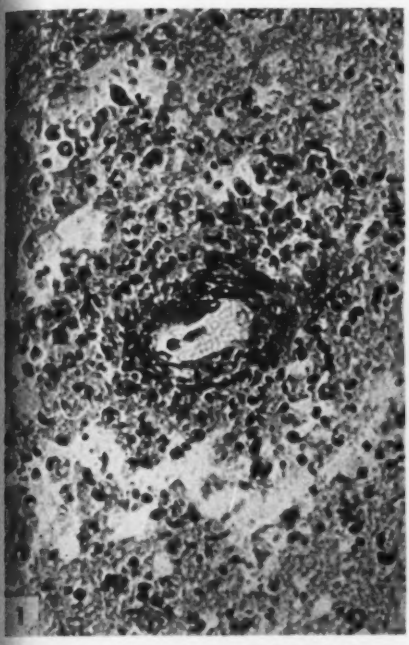
PLATE 117

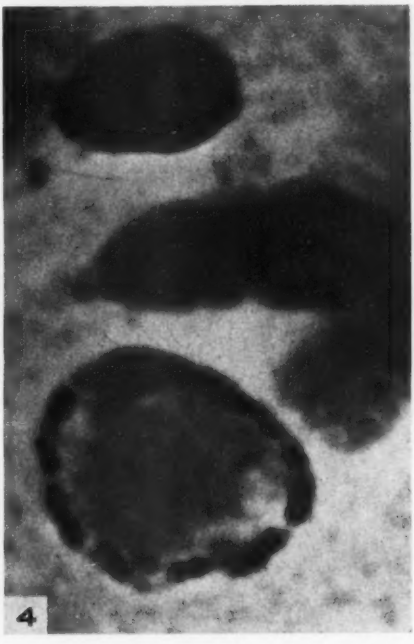
FIG. 1. Cerebrum. Area of necrosis infiltrated by polymorphonuclear cells and monocytes. × 450.

FIG. 2. Cerebrum. Blood vessel with marked lymphocytic cuffing. × 340.

FIG. 3. Cerebrum. Intranuclear inclusions in nerve cells. × 1700.

FIG. 4. Cerebrum. Single intranuclear inclusion. × 4230.





Kinney

Intranuclear Inclusions in Infancy

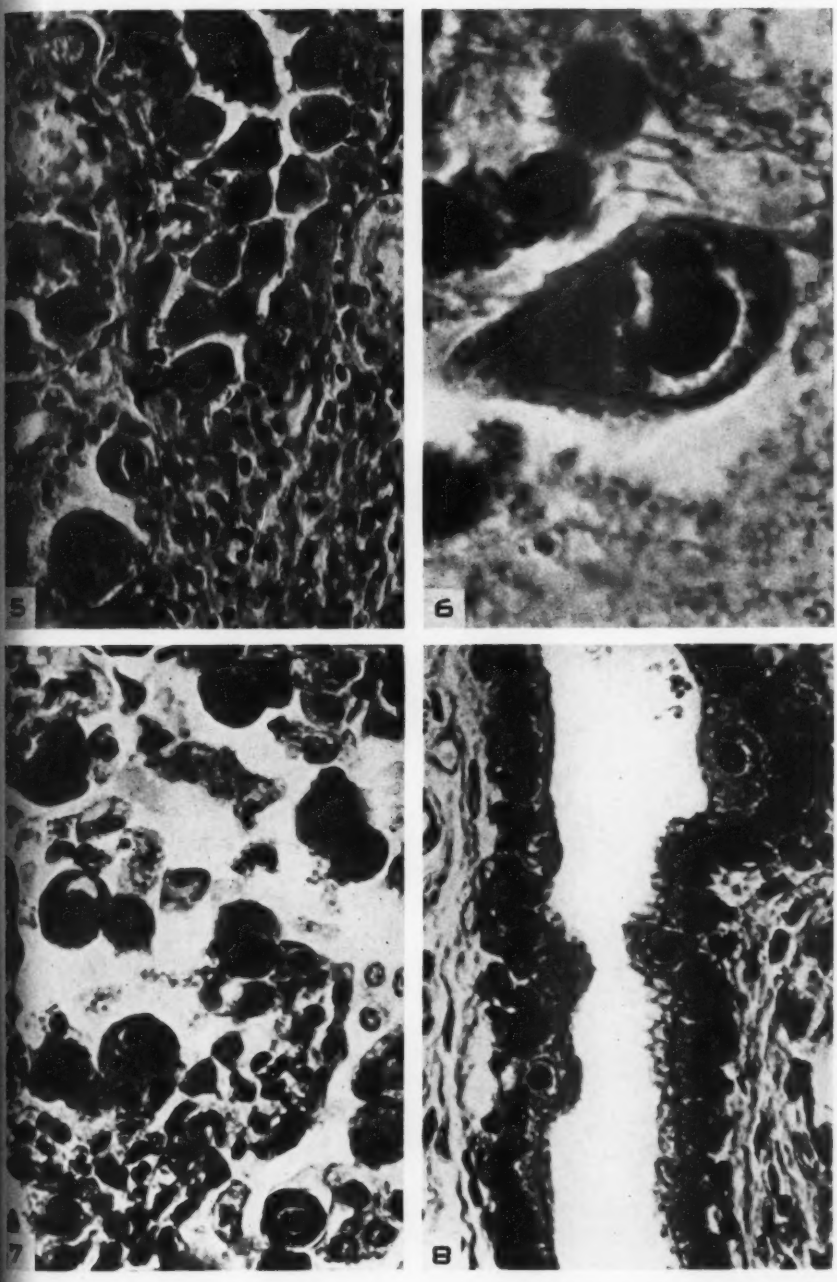
PLATE 118

FIG. 5. Kidney. Low-power photomicrograph illustrating both intranuclear and cytoplasmic inclusions. × 500.

FIG. 6. Cerebrum. Single intranuclear inclusion. × 1300.

FIG. 7. Lung. Many intranuclear inclusions. × 500.

FIG. 8. Salivary gland. Intranuclear inclusions in duct epithelium. × 500.



Intranuclear Inclusions in Infancy

PATHOLOGICAL CHANGES IN LISTERELLA INFECTION, PARTICULARLY OF THE EYE *

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During the course of an investigation on the genus Listerella [1-3] and its possible relation to infectious mononucleosis,[4, 5] a study has been made of the response of different tissues to experimental infection. The alterations stimulated by organisms of this group both in the peripheral blood and in the visceral organs of several species of animals have been described by other workers, and their reports have been reviewed recently by Conway.[6] In this communication, however, attention has been drawn to the lesion occurring in the eye; first, because a detailed histological description of the ophthalmic reaction has not heretofore been available and, secondly, because the corneal response provided suitable material for a study of capillary formation in infected tissue, avascular under normal conditions.

MATERIALS AND METHODS

Ophthalmic infections were regularly induced in rabbits and guinea pigs by simple instillation of several drops of a broth culture (18 to 24 hours) of the organism into the conjunctival sac.[1-3] Monkeys (*Macaca rhesus*), which respond irregularly to the inoculation performed in this way, were infected in some instances by intraconjunctival or subconjunctival introduction of organisms. Since the ocular infection was never fatal, animals were sacrificed at successive stages in the course of the disease and the ocular structures were removed for study. Palpebral tissue and the bulbus oculi were fixed in acetic Zenker's fluid; paraffin sections were prepared in the usual way and stained with hematoxylin and eosin. Pannus formation was further studied in whole mounts of the cornea prepared by a modification of the Spalteholz method.[7]

In addition, visceral lesions of rabbits, guinea pigs and mice infected by intravenous or intraperitoneal inoculation were studied. Tissues were obtained from animals which, for the most part, died of the infection or were sacrificed by intravenous injection of air. Paraffin sections were prepared from spleen, lymph nodes, liver and lungs which, after fixation in acetic Zenker's fluid, were stained with hematoxylin and eosin.

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Results

Visceral lesions encountered in generalized infection

Small quantities of *Listerella monocytogenes* injected into rabbits, guinea pigs and mice by any of several routes initiated a generalized infection, commonly terminating in death within a few days. The predominant changes consisted of areas of focal necrosis in the liver, spleen and lungs. In some animals the areas of necrosis suggested massive abscesses, but in every instance there was present an appreciable number of mononuclear cells, an unusual finding in abscesses caused by the more common pyogenic bacteria. A similar type of response to an overwhelming infection with Listerella has been reported previously by Bloom.[8, 9] A much milder mononuclear reaction was observed following injection of smaller doses of the organism. In the liver and lungs, moreover, small accumulations of lymphocytes and monocytes were encountered in the periportal connective tissue and in the peribronchial tissue, respectively.

Conway [6] has studied exhaustively the development and regression of the lesions in the spleen and lymph nodes of rabbits and guinea pigs following infection with this organism. Since the present observations are confirmatory of her findings, they may be briefly summarized. Within the first 48 hours the size of the malpighian bodies of the spleen was greatly increased by lymphocytes, monocytes and transitional forms. Within a comparatively few hours the cellularity of the white pulp decreased, but the red pulp now contained a great number of mononuclear cells; by 120 hours the spleen had returned to normal. The lymph node behaved similarly and showed a corresponding increase and proliferation of lymphocytes and monocytes. This activity persisted for 96 hours, but at 120 hours the organ regained its normal appearance.

An effort was made to determine whether the so-called "infectious mononucleosis cell" which Gall and Stout [10] described in lymph nodes excised from patients, occurred in the lymph nodes of rabbits experimentally infected with Listerella. Nodes were removed at different stages of infection from the omentum and inguinal region and occasionally from the axillary area. Paraffin sections were stained with phloxine and methylene blue as recommended by Gall and Stout. Despite careful and repeated search the "infectious mononucleosis cell" was never encountered in any of the material studied. Furthermore, the general appearance of the nodes showed none of the structural modifications observed by these authors in infectious mononucleosis. It would seem more likely, therefore, that the changes considered by

these authors as characteristic of infectious mononucleosis arise independently of infection with Listerella.

THE OPHTHALMIC REACTION

The ocular response of rabbits and guinea pigs to instillation of cultures of Listerella is essentially identical, and it is stimulated equally well by the different immunological types of the organism.[2, 3] Monkeys are more resistant to infection and display a milder reaction even when the bacteria are injected into the subcorneal tissue. Because of the similar response of different animal species, detailed discussion of the ophthalmic reaction will be limited to the observations made on infected rabbits.

Clinical Course of the Ophthalmic Reaction

The reaction was initiated by an intense conjunctivitis which appeared within 24 to 48 hours after the instillation of organisms and which was characterized by a thick, profuse discharge, chemosis, swelling of the lids and hyperemia of the palpebral and bulbar conjunctiva. A few days later the cornea also participated in the infection, exhibiting at first turbidity, then the development of capillaries, and eventually the formation of a heavy pannus which originated from the entire circumference and approached the center of the cornea. Figure 1 illustrates a typical involvement observed in the eye of a rabbit. Hypopyon, iritis and corneal ulceration have been encountered only exceptionally and, therefore, they should not be considered as characteristic of Listerella infection. The acute ophthalmic infection usually subsides in 2 to 3 weeks and complete clinical recovery occurs.

It may be pointed out that infection of the eye has not been encountered following inoculation by any route other than ocular. Conversely, generalization of the infection with septicemia and the development of lesions in the viscera have not been observed in animals with an ophthalmic reaction.

Daily blood counts were performed on a group of rabbits following ocular instillation of organisms in order to determine whether the hematological changes considered typical of infection with Listerella also occurred in this type of localized disease. Analysis of the data revealed no regular change in the quantity or quality of circulating white blood cells.

Anatomical Changes Observed in the Ophthalmic Reaction

Anatomical changes were studied in two types of material; *i.e.*, histological sections prepared in the usual manner, and corneal mounts prepared from eyes whose vessels had been injected with a colored

mass before fixation. Microscopical examination under low magnification of the latter type of preparation assisted in correlating the vascular reaction with the cellular changes encountered during the infection. The various phases of the reaction may be roughly grouped as early, intermediate and late stages.

Early Stage. Microscopical sections from the eyes of rabbits as early as 2 days after instillation showed a moderate accumulation of cells around the marginal vessels which are normally present at the sclerocorneal junction. Small lymphocytes comprised the majority of the infiltrating cells, but a few monocytes and polymorphonuclear leukocytes also were present. As the process advanced, the accumulation of cells about the vessels at the limbus increased. The majority of the cells observed on the fourth day were still mononuclear in character, consisting of small lymphocytes, monocytes and occasional macrophages; there were, however, many polymorphonuclear cells present at this stage. The invading cells were distributed centripetally along the lamellae of the edematous substantia propria toward the center of the cornea. Away from the sclerocorneal junction, the cells progressively decreased in numbers and the infiltration changed in character so that polymorphonuclear cells replaced mononuclear cells a short distance from the limbus. New vessels arising from the marginal vascular loops extended into the cornea. The corneal epithelium was thickened in places, especially over the large foci at the limbus, and many of the epithelial cells were in mitosis. In one section, polymorphonuclear leukocytes appeared to have invaded not only the substantia propria but also the overlying flattened corneal epithelium; it was evident that an ulcer was in process of formation in this region. A few mononuclear cells had invaded the sclera close to the sclerocorneal junction. The connective tissue fibers of the bulbar conjunctiva were widely separated by edema, and there was a scattering of mononuclear cells under the epithelium.

The *corneal mount preparations* made on the second day after inoculation revealed the penetration of the cornea by capillaries arising from both the marginal and conjunctival vessels. At this time the newly formed vessels were barely detectable in the gross even with the aid of a hand slit lamp or loupe. Examined under a dissecting microscope, the capillaries appeared as tiny, delicate loops returning to their original source, thus completing the circulation. The loops varied in number and were usually seen at different levels in the cornea. By the fifth day the vessels had penetrated three or four times farther than on the second day. The caliber of the vessels now varied greatly; the older ones were thicker, while the more recent ones were

thin and slender. Capillary loops of the variety seen earlier were still encountered but the connecting arches had disappeared from a number of vessels which now terminated as blind tubes. Many pin-point hemorrhages had spread into the interlamellar spaces from the distal ends of some of the vessels. These ecchymoses, however, were best seen by *in vivo* examination of the eye. As the infection progressed, looped vessels were seen to arise from corneal capillaries instead of from the conjunctival vessels. Capillaries were now observed not only connecting the vessels of the same level but also between vessels of adjoining levels. The offshoots of the conjunctival vessels were thicker at 5 days but they were not so numerous as earlier.

Intermediate Stage. Observations on the two types of preparation showed that the most severe lesions and the greatest development of the pannus occurred about 9 to 10 days after inoculation.

Microscopical sections revealed denser foci of mononuclear cells at the limbus, and polymorphonuclear infiltrations of the cornea were more intense than previously observed. In some instances a shallow ulcer was present on the corneal surface; it was characterized by complete loss of epithelium with a base formed by a collection of polymorphonuclear cells and débris. In other sections, the epithelial surface was intact but inflammatory lesions extended down through the cornea to the anterior chamber. The edema of the substantia propria was more marked. Mononuclear and polymorphonuclear leukocytes were observed throughout the substantia propria from the epithelium to the membrane of Descemet; the cells of the adjacent corneal endothelium appeared swollen and vacuolated.

The infection had spread down into the ciliary body; the iris was not invaded, but its vessels were dilated. In none of the sections was there evidence of an accumulation of cells or débris in the fluid of the anterior chamber. Structures of the posterior chamber showed no changes.

The epithelial cells of the bulbar conjunctiva were hydropic; the underlying collagenous tissue was edematous and lightly infiltrated by cells of the mononuclear and polymorphonuclear series.

About the twelfth day the intensity of the reaction began to diminish. There were fewer cells collected at the limbus, and there was only a slight infiltration of the cornea beyond this point. The polymorphonuclear leukocytes disappeared, leaving only foci of mononuclear cells. The vessels were now being resorbed.

Corneal mounts prepared from eyes removed on the ninth day clearly revealed the main vascular stems at the limbus. These were quickly lost in the dense capillary network which was characterized

by a tremendous degree of crowfooting at the terminus of most of the vessels. Arches and loops had almost entirely disappeared and the petechial hemorrhages in the corneal spaces were intensified. The capillary network began to recede at about the time when the cellular infiltration regressed. First, crowfooting disappeared and the capillaries appeared as long, slender vessels with a minimum of branching; then these decreased in number until only a few remained.

Late Stage. Microscopically, there was little evidence of the preceding infection 3 weeks after instillation of bacteria. At this time the cornea appeared to be almost normal; there were only small accumulations of mononuclear cells limited to the limbus; the recent vascular proliferation had disappeared; and the epithelium was now intact.

Corneal mounts likewise revealed a normal vascular bed in 3 to 5 weeks after infection.

Reaction of Periocular Structures. The palpebral conjunctiva, like the bulbar conjunctiva, showed the effect of the infection. During the first 5 days there was an infiltration of the epithelium and of the underlying tissue by polymorphonuclear and mononuclear cells. After this period the polymorphonuclear leukocytes disappeared, leaving a scattered collection of mononuclear cells. The connective tissue was edematous in the early stage. It is of interest to point out that healing of the conjunctiva by cicatrization has never been observed.

Discussion

The lesions encountered in this study in the spleen, liver and lung of rabbits, mice and guinea pigs infected with the genus Listerella are essentially identical with those described originally by Murray, Webb and Swann [11] and subsequently by other workers.[5, 9, 12, 13] In general, these consist of areas of focal necrosis scattered through the organs. When overwhelming doses of bacteria are injected, however, massive necrotic areas develop which differ from ordinary pyogenic abscesses only in the presence of a preponderance of mononuclear leukocytes.[5, 9] In the lymphoid tissue of the spleen an increase in the size of the malpighian bodies due to the proliferation of the mononuclear elements occurs first; then the bodies decrease in size and the mononuclear cells appear in the red pulp. A similar cycle is also observed in the lymph nodes.[6, 8, 9] In the present study of the lymph nodes there was no evidence of the "infectious mononucleosis cell" which Gall and Stout [10] described in lymph nodes taken from humans suffering from infectious mononucleosis. While histological observations on the central nervous system were not included in the animals

studied at this time, meningo-encephalitis has been reported in Listerella infections in man [14] and animals.[15]

Histopathological changes which develop in the eyes of rabbits and guinea pigs following instillation of Listerella organisms into the conjunctival sac have been studied. The early lesion consists of collections of mononuclear cells about the vessels of the limbus at the sclerocorneal junction. As the disease progresses, polymorphonuclear and mononuclear leukocytes invade the cornea and, to a lesser degree, spread into the sclera. The cornea becomes edematous and highly vascularized. Rarely, ulceration of the surface of the cornea occurs. The process reaches its peak about 9 days after infection, and then gradually recedes; the cornea generally returns to normal between the second and third week. Infection effected by instillation of Listerella on the uninjured cornea remains localized in the eye, and, conversely, parenteral injections of the organisms result in visceral lesions but never infect the eye.

Vascularization of the infected cornea is similar in most respects to the corresponding process observed in allergic inflammatory reactions,[7] or in trachomatous infection.[13] Vascular loops spring from the conjunctival and corneal vessels. These loops become separated at their centers, leaving stems from which blood extravasates. After several repetitions of loop formation and degeneration, the capillaries eventually complete the process of vascularization by throwing out crowfoot or arborescent terminations. The vascularization of the cornea is at its height about 10 days after infection and it requires 3 weeks or more for complete healing.

Summary

The histopathological changes in the ophthalmic response of several species of animals to infection with organisms of the genus Listerella have been studied. The ocular reaction after instillation of organisms is characterized by an inflammatory response in the conjunctiva and cornea without appreciable involvement in the deeper structures of the eye. In general, the cellular infiltration in the first few days consists principally of lymphocytes, but by the fifth day the proportion of polymorphonuclear leukocytes increases until these cells predominate by the tenth day. Edema of the involved structures is a prominent feature at the height of the infection. Vascularization of the cornea develops concomitantly with the other evidences of inflammatory response. The pathological changes rapidly recede and the eye generally heals without scarring 2 to 3 weeks after instillation of organisms.

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DESCRIPTION OF PLATES

PLATE 119

FIG. 1. Clinical appearance of rabbit eye 7 days following instillation of Listerella. Thick exudate, edematous and inflamed lids, and corneal reaction of marked clouding and massive vascularization are shown. × 3.

1

PLATE 120

FIG. 2. Cornea of rabbit 2 days after instillation of Listerella organisms. A small collection of cells is present around the marginal vessels of the sclerocorneal junction. Higher magnification shows these to be principally lymphocytes. Hematoxylin and eosin stain. × 38.

FIG. 3. Cornea of rabbit 5 days after infection. A marked increase in the cellular infiltration and in vascularization has occurred since the stage illustrated in Figure 1. Lymphocytes and mononuclear leukocytes still make up the bulk of the infiltrative reaction but polymorphonuclear leukocytes are beginning to appear. The lamellae of the cornea are spread apart by edema. Hematoxylin and eosin stain. × 38.

FIG. 4. Cornea of rabbit 9 days after infection. The accumulation of cells at the limbus is still dense and the infiltration has extended toward the center of the cornea. Polymorphonuclear leukocytes form the majority of the inflammatory cells. Many newly formed vessels accompanied by infiltrating cells occur throughout the width of the cornea. The width of the corneal substantia propria has increased greatly due to edema. Hematoxylin and eosin stain. × 38.

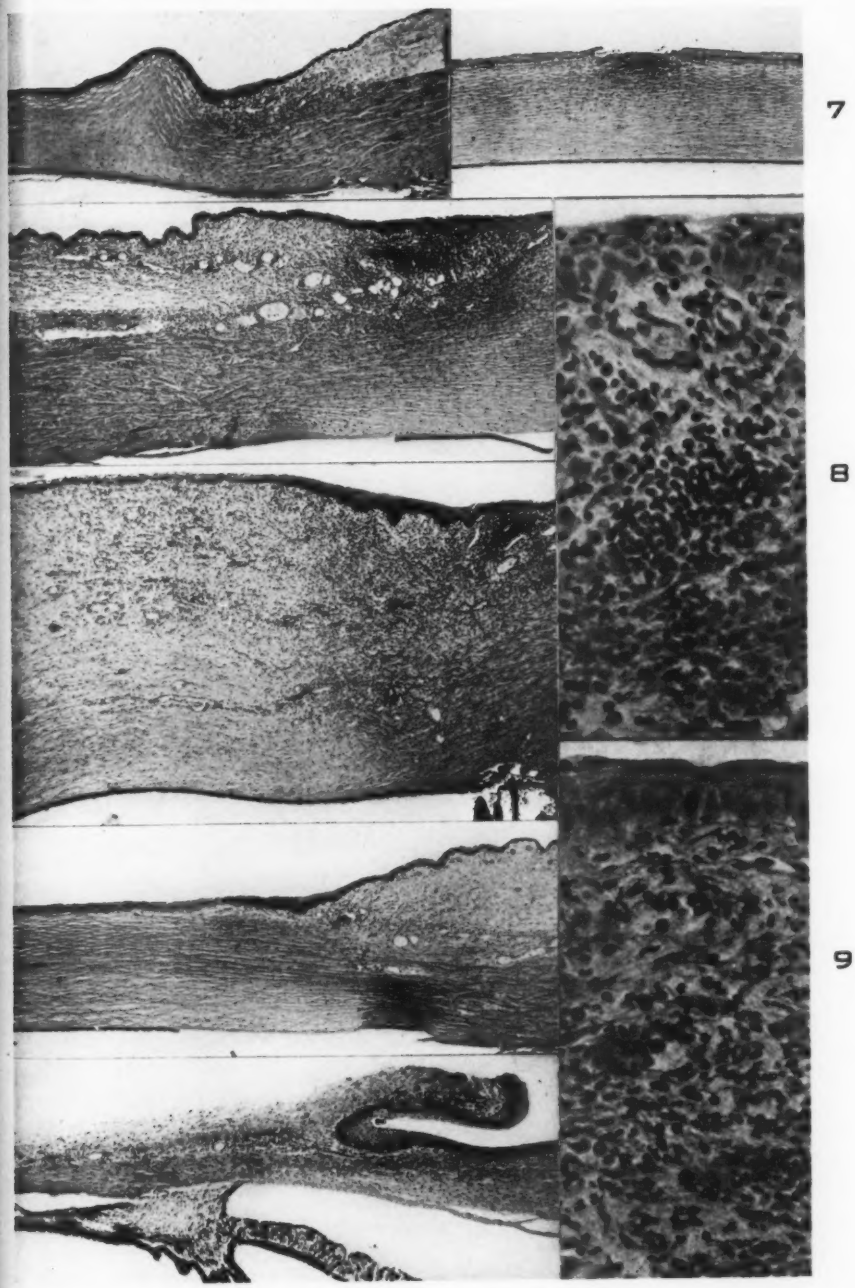
FIG. 5. Cornea of rabbit 21 days after infection. Only a slight collection of cells, principally lymphocytes, remains at the limbus. Most of the new vessels have disappeared. Hematoxylin and eosin stain. × 38.

FIG. 6. Cornea of guinea pig 5 days after infection. The reaction is similar to that observed in the rabbit. Hematoxylin and eosin stain. × 38.

FIG. 7. Cornea of rabbit 21 days after infection. A small, shallow ulcer is apparent. Hematoxylin and eosin stain. × 38.

FIG. 8. Cornea of rabbit 5 days after infection. Lymphocytes and monocytes are clustered about small vessels. Hematoxylin and eosin stain. × 300.

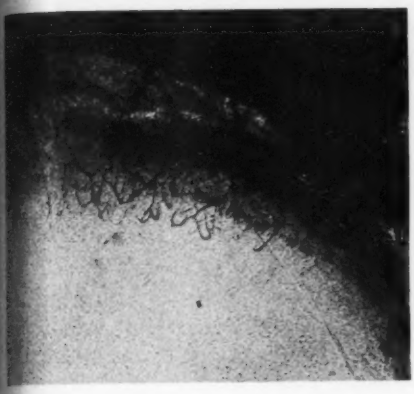
FIG. 9. Cornea of rabbit 9 days after infection. The increased vascularity is apparent. The majority of the infiltrating cells are polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 300.

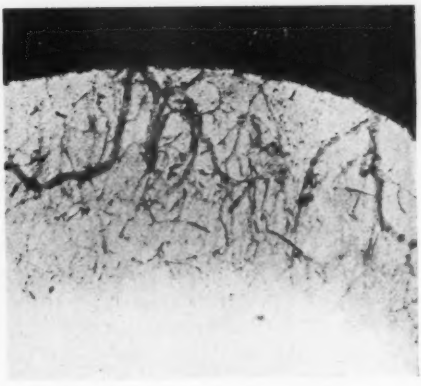


Julianelle and Moore Pathological Changes in Listerella Infections

PLATE 121

FIGS. 10–15. Progress of corneal vascularization in the rabbit as seen in corneal mounts. Figures 10 and 11 present the changes at 2 and 5 days. Figures 12 and 13, at 8 and 10 days and Figures 14 and 15 at 12 and 18 days following instillation, respectively.





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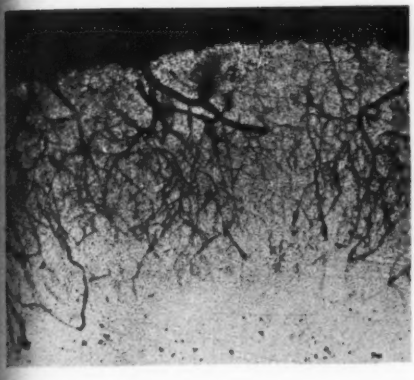

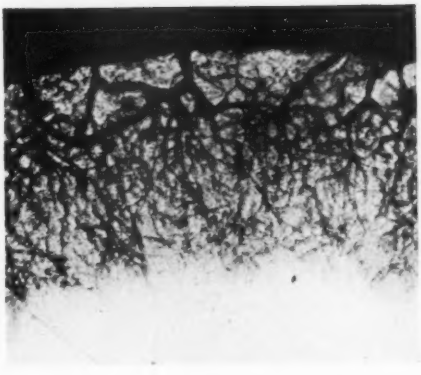

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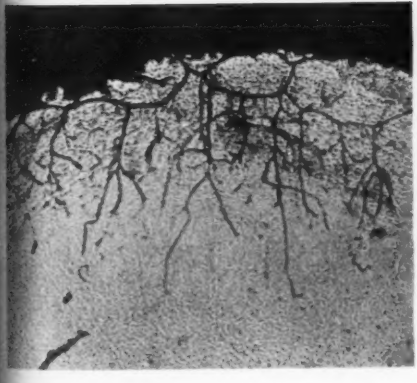

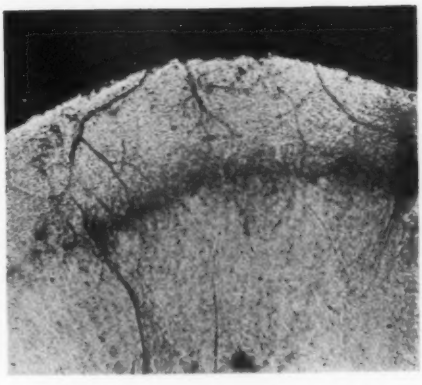

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THE CHORIOALLANTOIC MEMBRANE OF CHICK EMBRYOS AND ITS RESPONSE TO INOCULATION WITH SOME MYCOBACTERIA*

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The great value of inoculation of the chorioallantoic membrane of the developing chick since it was first described by Levaditi[1] in 1906, when he studied the spirillum of fowls, has been adequately proven and enhanced by Goodpasture and his associates. The successful work done by numerous workers with such disease incitants as viruses, Rickettsiae, bacteria, the spirochetes and fungi has stimulated the undertaking of further investigation, utilizing the chorioallantois.

The infected membrane lends itself easily and within a short time to a careful study of the histopathogenesis of the disease process. The possibility of day by day or even hour by hour examination of the lesion facilitates the tracing, microscopically, of the various types of cells that may become involved in the evolution of such a lesion. In like fashion, the method has made it a relatively simple matter to follow the fate of the organism and to note changes in morphology, especially of fungi.[2]

A group of microorganisms known as Mycobacteriaceae and often referred to as mycobacteria or *acid-fast organisms* is thought by some to be closely related to the fungi. As a consequence of the experiments on the inoculation of the chorioallantoic membrane of chick embryos with fungi, similar work was begun with several strains of mycobacteria for several reasons. In the first place, lesions produced by several of the acid-fast organisms are often confused with various mycotic granulomata. It was thought that this procedure might shed light on their differentiation. Secondly, as with fungi, the evolution of an experimental lesion, particularly of tuberculosis or leprosy—mycobacterial diseases—is exceedingly long, requiring from weeks to months. It was hoped, therefore, that the length of time required to produce an experimental lesion might be shortened to a few days by the use of the chorioallantois. Thirdly, it has been the desire of various workers in tuberculosis to find a means of determining virulence in a relatively short time by a simple method; and fourthly, it was thought that the use of this membrane might also make it possible to

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differentiate the various strains of mycobacteria by the type of reaction produced in the inoculated chorioallantois.

The published reports dealing with the inoculation of the chorioallantois with mycobacteria are few in number. Goodpasture and Anderson,[3] in 1937, obtained lesions with *Mycobacterium tuberculosis avium*. The lesions manifested themselves within the membrane up to the fourth day; then as nodules of the drying chorioallantois. Costil and Bloch,[4] in 1938, were also able to obtain nodular growths within 7 days using both human and avian strains of tubercle bacilli. In a preliminary report, I[5] described experimental inoculations of the chorioallantois with human and avian tubercle bacilli and with leprosy bacilli. This was followed[6] by a report which included the additional strains of bovine, fish and snake tubercle bacilli. The final paper to date, as far as is known, is by Emmart and Smith[7] who utilized the chick membrane for inoculation with three strains of human and one strain of bovine tubercle bacilli.

Materials and Technic

The technic of inoculation of the chorioallantoic membrane of the developing chick, generally considered as the Goodpasture-Buddingh technic, is an elaboration by Goodpasture of a technic described by Clark.[8] The technic was adapted by Woodruff and Goodpasture[9] to obtain lesions with fowlpox virus. Goodpasture and Buddingh[10] further developed the experimental procedure to its present-day method of practicability. As used here, the technic is essentially the same as that described by them and as followed in other publications.[4,6,11]

Various strains of acid-fast organisms from different sources, some of known virulence, others not well determined, have been used in several series, some over a period of approximately 2 years. Several hundred eggs were utilized. These organisms were the following:

1. *Mycobacterium tuberculosis hominis*. Virulent strain from Department of Bacteriology, Washington University School of Medicine; strain H160 from Dr. H. J. Corper; PN strain from U. S. Department of Agriculture; strain A 18 from Dr. Florence Seibert, Henry Phipps Institute; virulent strain from Department of Bacteriology, St. Louis University Medical School; and nonclassified strains from St. Louis City Health Laboratories.

2. *Mycobacterium tuberculosis bovis*. Virulent strain from Dr. H. J. Corper; strain 854 from U. S. Department of Agriculture; bovine "S" strain from Dr. Florence Seibert, Henry Phipps Institute.

3. *Mycobacterium tuberculosis avium*. Strain from Department of Bacteriology, Washington University School of Medicine; strain 14,619

from Dr. H. J. Corper; V. E. strain from U. S. Department of Agriculture; strain no. 36 from Dr. Florence Seibert, Henry Phipps Institute.

4. *Mycobacterium marinum* (fish). Strains nos. 1 and 2 from Dr. J. D. Aronson, Henry Phipps Institute.

5. *Mycobacterium thamnopheos* (snake). Strains nos. 1 and 2 from Dr. J. D. Aronson, Henry Phipps Institute.

6. *Mycobacterium leprae muris* (rat). Rats with lepromata obtained from Dr. E. V. Cowdry, Department of Anatomy, Washington University School of Medicine. Bacilli were expressed from lepromata. The lepromata varied in age from 8 to 10 months.

Eggs were incubated 12 days and then inoculated. Eggs incubated 10 days were found to be less hardy than those incubated 12 days and, therefore, were not used in subsequent series. In some series eggs were incubated 13 days. These served the purpose equally as well, but did not allow as much time for the development of lesions.

The inoculations in several series consisted of a loopful of organisms implanted on the ectodermal surface of the chorioallantoic membrane. In other series, amounts of 0.1 mg. to 1.0 mg. of bacilli were planted on the membrane. Best results were obtained with mass implantations and with the higher concentrations of bacilli.

Following inoculation, the eggs were incubated in a bacteriologic incubator. After 5 days in some series and after 6 days in other series, the inoculated membranes were removed, fixed in Zenker's solution (with 5 per cent acetic acid), embedded in paraffin and sectioned. The sections were stained for acid-fast organisms and duplicates stained with hematoxylin and eosin. These slides were studied to determine the character of the lesions.

Experimental Data

The experimental results obtained are encouraging and point to a number of interesting and distinguishing features which characterize the particular organism involved.

Mycobacterium tuberculosis hominis

Eggs were incubated 12 or 13 days and fixed 5 or 6 days after inoculation. Many of the embryos died. A small number in each series remained alive, but apparently were considerably weakened. These showed nodules on the membrane with infiltration and thickening of the membrane. The nodules were either discrete or confluent, with a peripheral opacity of light grayish infiltrate.

Interspersed among the bacilli of the inoculum were seen a large

number of monocytes and leukocytes, the latter usually degenerating. At the margin of the inoculum was a layer of giant cells of the Langhans type. The ectoderm in this area underlying the inoculum seemed to have lost its normal character and was replaced by monocytes, degenerate polymorphonuclear leukocytes and the Langhans type of giant cells. In other areas, the hyperplastic ectoderm showed marked thickening, with the entire surface appearing flattened or in the process of desquamation.

In the mesoderm the reaction varied according to the virulence of the organism. With organisms showing decreased virulence the process simulated a mild foreign body reaction with very little cellular response except for a few mononuclears and some leukocytes which surrounded the bacilli. In other cases, bacilli were engulfed by a small giant cell, with very little other cellular response. Virulent organisms rapidly invaded the mesoderm and formed definite tubercles showing caseation necrosis. The mesoderm showed marked enlargement or swelling which was particularly apparent where the organisms had invaded the layer (Fig. 1).

The development of tubercles with virulent organisms seemed to follow a pattern or sequence of events. In the first place, organisms planted on the ectoderm either invaded the mesoderm as single cells which multiplied to form clumps or bundles of parallel cells, usually rods, with some showing beaded forms (Fig. 2); or, and this seemed to be the customary method, they were taken up by wandering cells (clasmatocytes, phagocytes) of the ectoderm and carried into the mesoderm. Secondly, the wandering cells lodged in the mesoderm and the bacilli multiplied, causing the host cells to become swollen and then to rupture, liberating the organisms. The invasion by the bacilli was followed by a rapid formation or calling forth of mononuclear or epithelioid cells and monocytes to the site of the bacilli, which in time was followed by a marked fibroblastic response, interspersed with some eosinophil-like cells (Fig. 3). The evidence of rapidity of cellular response was indicated by the large number of mitoses of the epithelioid cells which were seen in the area of the organisms (Fig. 4). Just as there was an increase in the number of epithelioid cells and fibroblasts, so was there likewise an increase in the number of capillaries. The capillaries were most numerous in the area of greatest activity. The capillary walls seemed thinner and eventually ruptured and formed the central area of necrosis and perhaps caseation. The resulting tubercle was of the "soft" type (Fig. 5).

Early tubercles showed an accumulation of mononuclear cells engulfing bacilli. The mononuclear cells apparently fused, producing

irregular giant cells with the nuclei scattered particularly around the periphery, with an opaque central area (Fig. 6). As the tubercle became older, the mononuclear cells extended outward from the central mass (palisading) and formed the giant cells which were characteristic of these tubercles.

There was marked hypertrophy of the ectoderm which manifested itself in the form of extensions into the mesoderm. These could be seen usually as "pearls," either detached from the ectodermal layer or, as is most likely, only apparently detached due to sectioning, whereby cross section made them appear as balls of epithelial cells in pearl formation. Occasionally the twisted connection could be seen clearly. Tubercle bacilli were found in the core of these "pearls" (Fig. 7). This indication of hyperkeratinization was not particularly marked, however, in the membranes inoculated with human tubercle bacilli.

The entoderm seemed little affected, if at all.

The significant points can be considered briefly as marked invasion of the mesoderm with virulent organisms; definite tubercle formation with perhaps caseation and necrosis to form "soft" tubercles; marked infiltration of the mesoderm with epithelioid cells, fibroblasts, monocytes and eosinophil-like cells accompanied by bacilli which may be found isolated, or in clumps, surrounded by various cells or engulfed by macrophages or giant cells of the Langhans type.

With less virulent bacilli the significant points are that the organisms do not invade the mesoderm rapidly; the mesodermal reaction is mild with few epithelioid cells, fibroblasts, monocytes, or giant cells; the organisms may appear freely distributed or they may be engulfed by macrophages or by giant cells of the Langhans type with little or no accompanying cellular reaction.

Mycobacterium tuberculosis bovis

Eggs were incubated 12 or 13 days and observed 5 or 6 days after inoculation. Many of the embryos died. The membranes of the living embryos showed, grossly, discrete or confluent plaques and nodules with an opacity of the membrane. The membrane appeared thickened in the area of marked involvement.

The first evidence as a result of inoculation with bovine tubercle bacilli was a nodular thickening of the ectoderm. The marked ectodermal hyperplasia resulted in a heaping up of the ectodermal layer with cornification or stratification of the outermost area. This reaction simulated parakeratosis in some areas while in others the appearance was that of hypertrophy and hyperkeratinization. As further evidence of this reaction, the ectodermal invagination into the mesoderm was

particularly well noticed in the formation of large numbers of "pearls," formed especially in the mesodermal area just underlying the affected ectoderm and extending through the mesoderm (Fig. 8).

In other areas of the membrane, the ectoderm appeared to have been separated from the rest of the membrane, as though by fluid. In still other areas, especially where there was marked reaction, the ectoderm was replaced by many monocytes, polymorphonuclear leukocytes, epithelioid cells, some basophilic leukocytes and blood cells.

Invasion of the mesoderm by the bacilli was more apparent with a virulent strain. The number of organisms found in the mesoderm was in no case as great as in the same layer in membranes inoculated with virulent human tubercle bacilli. The mesodermal response, although made apparent by the presence of epithelial "pearls," showed little other activity except for swelling in affected areas and for the formation of scattered early tubercles (Fig. 9). These tubercles differed somewhat from those seen with inoculations of human strains. They were made up essentially of mononuclear or epithelioid cells, some fibroblasts, leukocytes and giant cells (Fig. 10). In some areas showing bacilli, "pearls" were found which contained organisms apparently from the ectodermal inoculum (Fig. 11). The presence of bacilli in these structures would seem to make them macrophages, no doubt of epithelial origin. Scattered freely among these cells or engulfed by the macrophages were the bacilli, which appeared as rods, either singly or in clumps, chiefly the latter. Capillaries seemed increased in number, extending particularly to the ectodermal surface and at the upper portion of the mesoderm at the epidermal junction. Capillaries also seemed more abundant in the vicinity of the tubercles. Necrosis was slight or lacking, and in no case marked. Caseation was not noticed in any of the tubercles seen.

The entoderm showed little response to the invading bacilli.

Tubercle formation as found in membranes inoculated with bovine tubercle bacilli is, therefore, quite different from that produced by human tubercle bacilli in that the tubercles lack caseation and necrosis is slight or lacking; epithelial hyperplasia is more apparent, manifesting itself as marked ectodermal proliferation into the mesoderm in the form of ectodermal "pearls" as seen on cross section.

Mycobacterium tuberculosis avium

Eggs were incubated 12 days and observed 5 days after inoculation. A number of eggs were examined on the third and fourth day after inoculation. The embryos became weak on the third day, with several dying on the fourth day and most of them dying on the fifth day.

The reactions obtained with avian tubercle bacilli varied according to the virulence of the strain of organism used. The differences in membranal response, however, were sufficiently characteristic to make differentiation possible.

Grossly, the response of the membranes to virulent avian tubercle bacilli have already been described by Goodpasture and Anderson.[3] The membranes first showed a thickening, becoming clear and gelatinous, with the lesions apparently within the membrane. After about 5 days the membranes became dry and showed nodular formations. Microscopically, the finding of organisms in foci within the mesoderm confirmed this observation (Fig. 12).

Organisms planted on the membrane were rapidly taken up by mononuclears or phagocytes, perhaps clasmatocytes, and rapidly penetrated into the mesoderm where they set up foci. In these membranes, the ectoderm showed little or no inflammatory response, except that some bacilli were seen in ectodermal cells. The mesodermal response was not outstanding except for a proliferation of fibroblastic cells, within some of which bacilli were found. The number of bacilli in the macrophages or in the fibroblasts varied. In some there were a few rods while in others the host cell was packed (Fig. 13). In the mesoderm, no extracellular bacilli could be noted and this simulated closely the reaction seen in leprosy where the lepra bacilli are taken up by macrophages termed "foam cells." The bacilli were chiefly of the rod type, but coccoid forms were also seen and these were all acid-fast. Giant cells have not been found where virulent organisms were inoculated, but some epithelioid cells were apparent.

Avian bacilli that were somewhat less virulent produced a slightly different picture. The ectoderm became involved where the bacilli were planted. There was an ectodermal response with some hyperplasia and resultant inflammation. The bacilli were taken up by macrophages. The area was infiltrated with monocytes and epithelioid cells and red blood cells could also be seen. The macrophages loaded with bacilli penetrated into the mesoderm and occurred in clumps or were seen at a distance from the main group of loaded phagocytes (Fig. 14). The number of capillaries was increased in the areas of greatest response. Some capillaries were surrounded by monocytes, although the reaction was not intense. Certain large capillaries as well as other large blood vessels showed, in addition to numerous red blood cells, groups of monocytes. Fibroblasts were seen in large numbers but these were found chiefly in zones at the periphery of the lesion. Giant cells were not seen in any number in the mesoderm; however, small giant cells of the Langhans type could be seen at the base of the inocu-

lum on the ectoderm. These formed a layer and were made up apparently of groups of mononuclear cells with the nuclei arranged peripherally, acting perhaps as foreign body giant cells (Fig. 15).

Where the organisms had penetrated in large clumps, no doubt part of the inoculum, with many bacilli embedded in an opaque substance, there was also a heavy infiltrate of monocytes, leukocytes and epithelioid cells. In this case, the clumps of organisms in the opaque matrix were surrounded by epithelioid cells. This induced the formation of giant cells of the Langhans type and was, therefore, a foreign body reaction, in which the foreign substance was the clump, with the epithelioid or mononuclear cells fusing to form large macrophages. Here there was also an increase in capillaries and polymorphonuclear leukocytes. Fibroblasts were also seen at the periphery of the mesodermal involvement, as well as basophilic leukocytes. The whole appearance was that of a foreign body granuloma. The entoderm was not affected.

With mild or avirulent organisms the reaction was almost entirely limited to the ectodermal implant with a marked infiltrate of monocytes and leukocytes. Invasion of the mesoderm by bacilli was mild and the mesodermal reaction was also mild except for some monocytes.

The chief points to be considered in chorioallantoic inoculation with avian tubercle bacilli are, first, the fact that the bacilli are taken up by macrophages showing intracellular bacillary growth with no extracellular bacilli in the invaded mesoderm. The reaction of the mesodermal layer to this invasion is not remarkable, showing many monocytes, leukocytes, epithelioid cells and an increase in number of capillaries. Giant cell formation is chiefly a foreign body reaction and is not as noticeable in these tubercles as in those formed as a result of inoculations with human tubercle bacilli.

Mycobacterium marinum

Eggs were incubated 12 days and observed 6 days after inoculation. Many of the embryos were dead, but grossly the membranes of the live embryos showed discrete or confluent gray plaques. The membranes seemed thickened and somewhat gelatinous in appearance. The reaction appeared to be within the thickened membrane to a greater extent than on the surface. However, there were a number of small, discrete plaques which were seen as small, raised nodules on the membrane.

Microscopically the fish tubercle bacilli produced a striking picture of rapid invasion and granulomatous response. Where the organisms had come in contact with the ectoderm to form nodules, the ectoderm was hyperplastic, thickened and raised, showing a parakeratotic type of response as well as keratinization. In other areas, particularly where

there was marked reaction, the ectoderm was either necrotic or ulcerating with an infiltrate of many monocytes, or it was separated from the mesoderm. Hyperplasia of the ectoderm was further emphasized by the formation of epithelial "pearls" which extended into the mesoderm. In the cores of some of these were found *Mycobacterium marinum* (Fig. 16).

Where the mesodermal reaction was most marked, the membrane was greatly thickened. In this case the rapidly developing granulomatous reaction presented several outstanding characteristics which easily separated the lesion from that caused by either human, bovine or avian tubercle bacilli. In the first place, the number of polymorphonuclear leukocytes was surprisingly great. These cells were seen scattered through the affected mesoderm, either singly or in clumps or groups forming microscopic abscesses. There were also basophilic leukocytes in varying numbers. Monocytes were also seen throughout the membrane and these, too, were found in clusters.

Another outstanding phenomenon was the presence of many groups of epithelioid cells, monocytes, fibroblasts and polymorphonuclear leukocytes, interspersed among which were bacilli occurring either singly or in clumps (Fig. 17). In the center of these groups there were capillaries which were apparently affected by the reaction since many showed broken walls and an infiltrate of monocytes and leukocytes. The capillaries throughout the affected area were greatly increased. Giant cells were not seen, but the groups of cells undoubtedly represented tubercle formation.

Acid-fast organisms were found chiefly as long rods, beaded, occurring freely, singly, or in clumps and usually within the group of cells.

Serial passage of ground-up infected membrane from egg to egg showed on the fourth passage a rapid invasion of the mesoderm with an accompanying monocytic and leukocytic infiltrate. The organisms apparently multiplied rapidly since they were seen in large clumps. The ectodermal reaction was likewise noticeable, but did not show marked hyperplasia. The reaction apparently was chiefly in the mesoderm (Fig. 18).

The entoderm appeared to be not affected.

The salient features of membranes inoculated with *M. marinum* are, briefly, a rapid invasion of the membrane, particularly the mesoderm; a granulomatous response with the presence of many polymorphonuclear leukocytes, monocytes and epithelioid cells; marked reaction involving the capillaries; the occurrence of many acid-fast organisms, either singly or in clumps, and no evidence of giant cell formation.

Mycobacterium thamnopheos

Eggs were incubated 12 days and observed 6 days after inoculation. Some of the embryos died, but a number were alive.

Grossly, the membranes showed grayish nodules or plaques which were spread over the membrane. The membrane was thickened in these areas and showed inflammation.

Microscopically, there were marked inflammatory changes in the ectoderm as well as in the mesoderm. The ectoderm was irregular, necrotic and infiltrated with monocytes and leukocytes. The appearance was that of an ulcer. Where the ectoderm was not destroyed, it was thickened, and showed an inflammatory reaction.

The presence of giant cells was a remarkable feature in these membranes. These were formed in the ectodermal reaction at the base of the inoculum, many fusing to form tubercles. They were seen in large numbers and extended into the mesoderm where they also fused to form tubercles (Fig. 19). The cellular reaction was notably of fibroblasts. These were seen in large numbers, but there were also some basophilic leukocytes and mononuclears. The number of capillaries was greatly increased. There was a marked reaction around the capillaries with many fibroblasts and polymorphonuclear leukocytes. A number of the capillaries had apparently ruptured since red blood cells could be seen scattered throughout the mesoderm.

Mycobacterium thamnopheos is difficult to stain in sections; consequently it was not possible to determine how far the organism had penetrated into the mesoderm. However, the marked response of the mesoderm and the finding of giant cells and groups of inflammatory cells deep in this layer would seem to indicate that the organism had been able to cause damage deep in the mesoderm where it no doubt invaded (Figs. 20 and 21).

The entoderm showed some hyperplasia in the area underlying the marked mesodermal and ectodermal response.

Of chief interest in membranes inoculated with *M. thamnopheos* is the great number of giant cells, many of which have fused to form tubercles, some showing caseation. The fibroblastic response is remarkable as is the activity in and around the capillaries.

Mycobacterium leprae muris

Eggs were incubated 12 and 13 days and observed 6 days after inoculation. Most of the embryos died, with the remaining live ones extremely weakened.

Macroscopically, the membranes appeared thickened, showing

opaque to grayish yellow areas, plaques and nodules. The general appearance indicated an inflammatory response of the membrane.

Microscopically, the ectoderm, in the area inoculated with organisms expressed from rat lepromata, was apparently disintegrated and invaded by many monocytes and macrophages or epithelioid cells. The inoculum with the resulting reaction made the lesion appear nodular, which may be compared with a nodular leproma (Fig. 22). At the margins of the lesion the ectoderm was thickened, with some hyperplasia, as evidenced by the presence of "pearls" in the adjacent mesoderm (Fig. 23).

At the junction of the inoculum and the affected mesoderm could be seen giant cells made up of epithelioid cells within which there were many bacilli. Fibroblasts extended along this area and appeared to be penetrating the inoculum. Capillaries were also found in larger numbers along the upper portion of the mesoderm, with many present in the deeper mesoderm. Polymorphonuclear leukocytes were also seen in large numbers. All of these cells were more numerous in the area underlying the inoculated ectoderm.

Of chief importance was the presence of large numbers of large phagocytes or macrophages with an eccentric nucleus. These cells, when stained with hematoxylin and eosin, appeared somewhat vacuolated (Fig. 24). When colored with carbol fuchsin they were found to be loaded with lepra bacilli. Stained in this manner, many of these phagocytizing cells showed large vacuoles, with the organisms filling the cell and causing it to become swollen. Formations of various types were seen in these cells. Semilunar arrangements and the formations termed globi were also made out. These "lepra" or "foam" cells are actually phagocytes (Fig. 25).

A striking resemblance is seen between inoculations of avian tubercle bacilli and those of lepra bacilli on the chorioallantoic membranes. The bacilli in both cases are chiefly intracellular. The chief difference, however, is in the type of phagocytizing cell. In the former the phagocyte is an epithelioid cell; in the latter it appears to be a specialized "lepra" cell. A nodular reaction is another important feature of membranes inoculated with *Mycobacterium leprae muris*.

Discussion

The inoculation of the chorioallantoic membrane of the developing chick with acid-fast bacilli is a new approach to the study and determination of the rôle played by these organisms in infectious processes. The relative simplicity of the technic lends itself easily to its application in experimental pathologic problems. But what is more important

is the well controlled test-subject as used in the procedure. The fertilized egg is easily controlled, both as to strain and temperature conditions. Food supply is eliminated as a factor. The possibility of existing or pre-existing infections can be ruled out as complicating the picture produced by the inoculum. Of equally great importance is the low cost per egg as compared with the cost of laboratory animals.

The study described here is concerned chiefly with the results when live mycobacteria are inoculated in the chorioallantois. Several important points have been described. In the first place, the histopathogenesis of these acid-fast bacterial diseases was easily traced. By this study the type of reaction produced by the various strains of bacilli could be differentiated. Certainly, virulence can be determined without difficulty. The rapid formation of tubercles with virulent strains definitely distinguishes these organisms from the nonvirulent or less virulent strains where tubercles are either of slow development and few, or lacking. The concerted action on the ectoderm in the inoculations of the bovine strain as compared with the mesodermal response in the membranes inoculated with human strains is an important differential point. The exclusively intracellular development of avian tubercle bacilli, their rapid spread into the mesoderm as seen in virulent strain inoculations and the different type of cellular response help easily to distinguish this organism from both human and bovine strains.

The rapidity in the formation of tubercles with virulent strains of tubercle bacilli in the short space of 5 or 6 days and perhaps in less time, when compared with the weeks or months necessary for the formation of tubercles in laboratory animals, favors the egg technic. These are only a few of the advantages. What perhaps appeals greatly to the technician is the finding of a method for the rapid identification of strains of tubercle bacilli and for the determination of their virulence. This can be done in the chorioallantois since the type of reaction and the intensity of the response are adequate for this purpose.

The results of inoculation of fish and snake tubercle bacilli further emphasize the value of the chorioallantois, for in them we have organisms occurring in cold-blooded animals which are able to produce definite lesions in a warm-blooded test-subject. This certainly opens the path for further experimental work with these acid-fast organisms. Again, the inoculation of the rat leprosy bacillus and the resultant lesion and response present a microscopic picture so characteristic of the spontaneous lesion that here, too, the path to further investigation—both with rat and human leprosy—is cleared of a number of obstacles. One of the chief problems of investigative work is the element of time. With the egg membrane this is cut down to an almost insignificant factor.

This work makes possible the investigation of the action of drugs or chemical fractions of organisms in inhibiting or enhancing the growth of the bacilli and thus determining the value of such agents in combating the disease.

SUMMARY

1. Strains of human, bovine, avian, fish and snake tubercle bacilli and of rat leprosy bacilli have been inoculated in the chorioallantoic membrane of the developing chick.

2. The histopathogenesis of the lesion produced by these various acid-fast organisms has been studied and differentiated.

3. The type of reaction and the degree of response of the membrane to the inoculum serve to determine virulence in a relatively short time.

4. The possibility of further work on methods of study, including the action of drugs or chemical fractions of the bacilli, are suggested.

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DESCRIPTION OF PLATES

PLATE 122

FIG. 1. Section through membrane inoculated with *Mycobacterium tuberculosis hominis*. Marked infiltration of mesoderm, with tubercle formation. Acid-fast stain. × 85.

FIG. 2. Group of bacilli in center of early tubercle. *Mycobacterium tuberculosis hominis*. Acid-fast stain. × 1025.

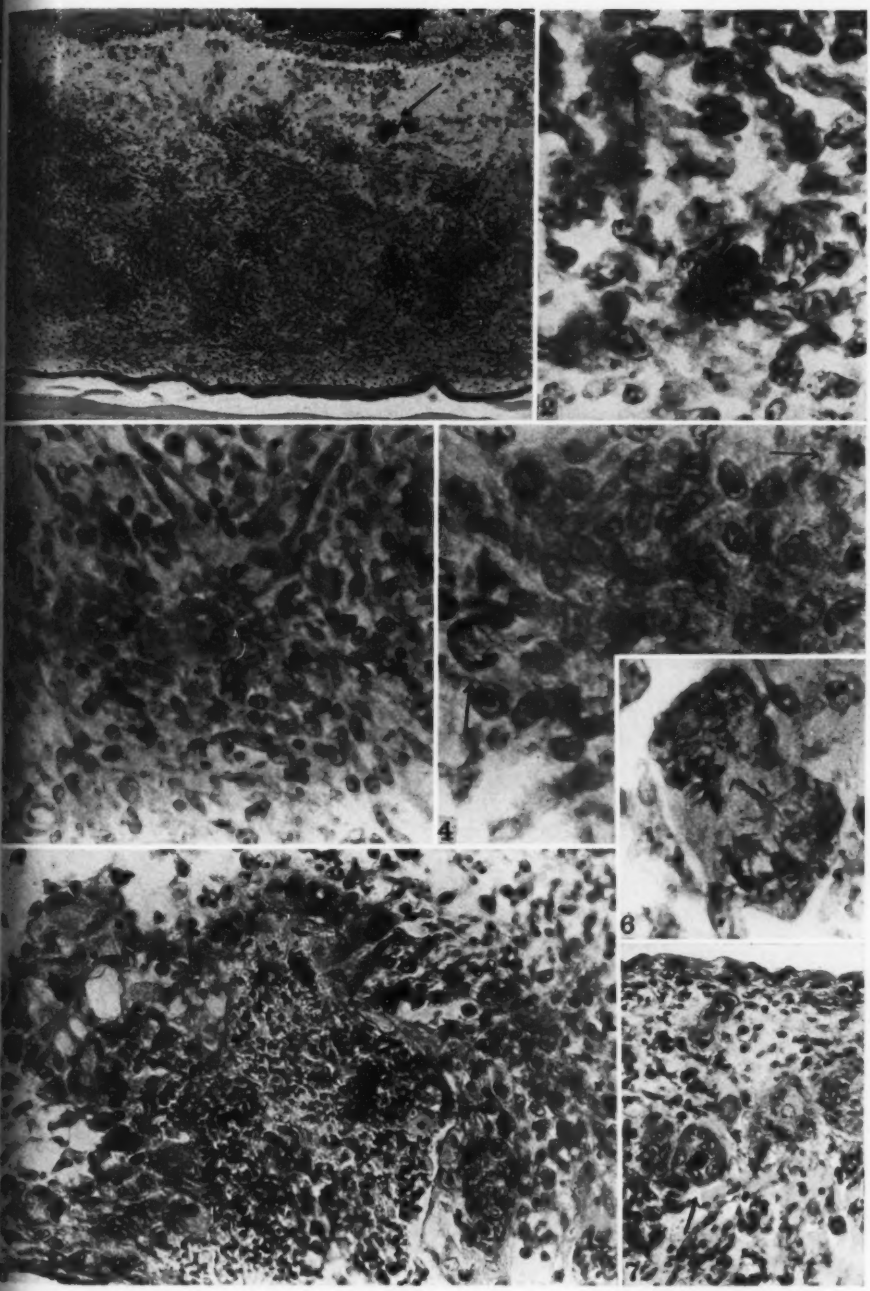
FIG. 3. Center of early tubercle formation. *Mycobacterium tuberculosis hominis*. Acid-fast stain. × 525.

FIG. 4. Mitotic cells in area of response to bacilli. *Mycobacterium tuberculosis hominis*. Acid-fast stain. × 920.

FIG. 5. Tubercle formation. Bacilli and a central area of necrosis are seen. *Mycobacterium tuberculosis hominis*. Acid-fast stain. × 420.

FIG. 6. Engulfed bacilli of *Mycobacterium tuberculosis hominis*. Acid-fast stain. × 900.

FIG. 7. *Mycobacterium tuberculosis hominis* in core of epithelial "pearl." Acid-fast stain. × 365.



Moore Mycobacteria in Chorioallantoic Membrane

PLATE 123

FIG. 8. "Pearl" formation in membrane inoculated with *Mycobacterium tuberculosis bovis*. Acid-fast stain. × 110.

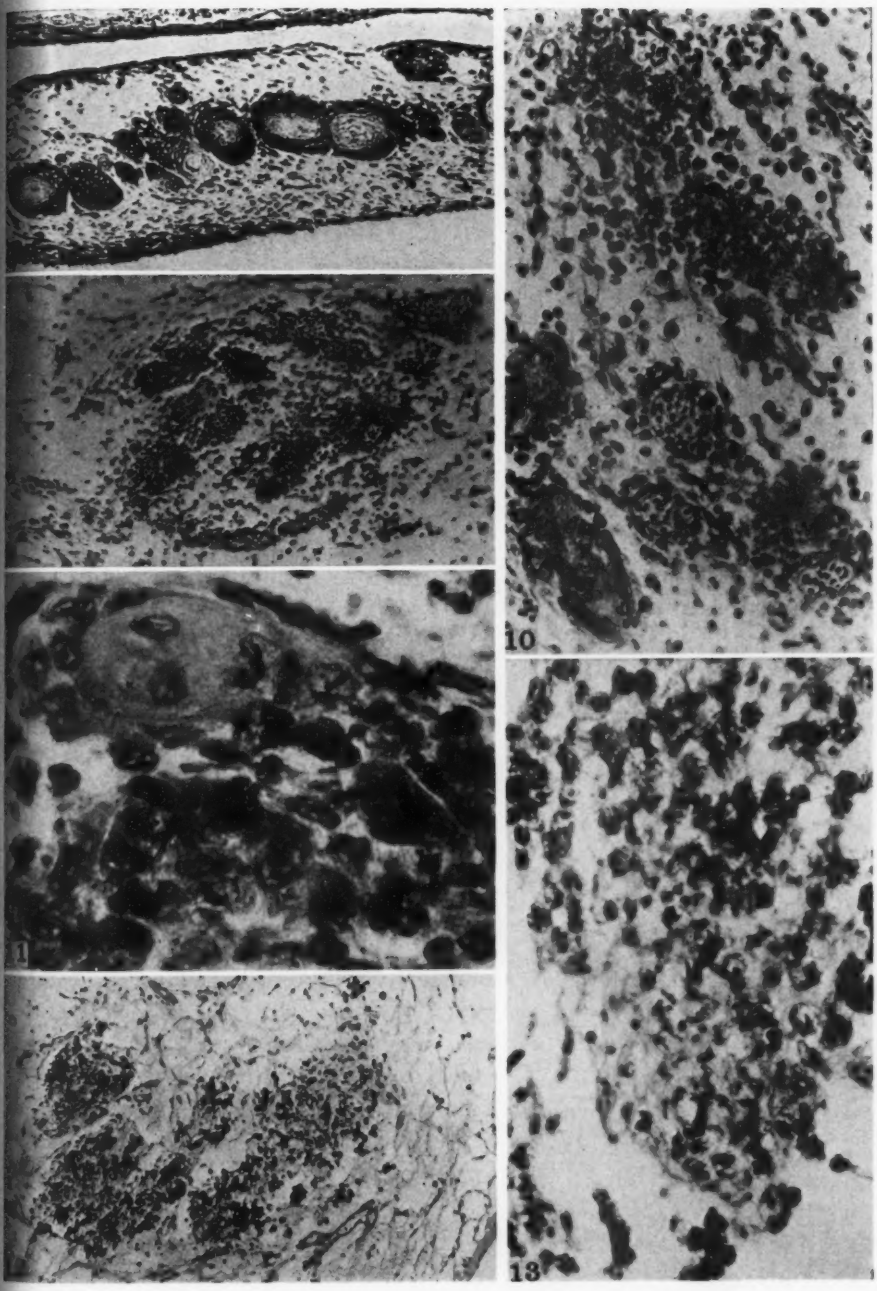
FIG. 9. Tubercle formation caused by *Mycobacterium tuberculosis bovis*. There is an increase in the number of capillaries. Acid-fast stain. × 145.

FIG. 10. Tubercle formation by *Mycobacterium tuberculosis bovis*. Giant cells, capillaries and cellular infiltrate are shown. Acid-fast stain. × 350.

FIG. 11. Tubercle caused by *Mycobacterium tuberculosis bovis*. Note "pearl" and numerous bacilli. Acid-fast stain. × 955.

FIG. 12. Mesodermal nodule showing numerous virulent bacilli of *Mycobacterium tuberculosis avium*. Acid-fast stain. × 95.

FIG. 13. Intracellular virulent bacilli of *Mycobacterium tuberculosis avium* in mesodermal nodule. Acid-fast stain. × 470.



Moore Mycobacteria in Chorioallantoic Membrane

PLATE 124

FIG. 14. Phagocytized bacilli of a less virulent strain of *Mycobacterium tuberculosis avium*. Acid-fast stain. × 900.

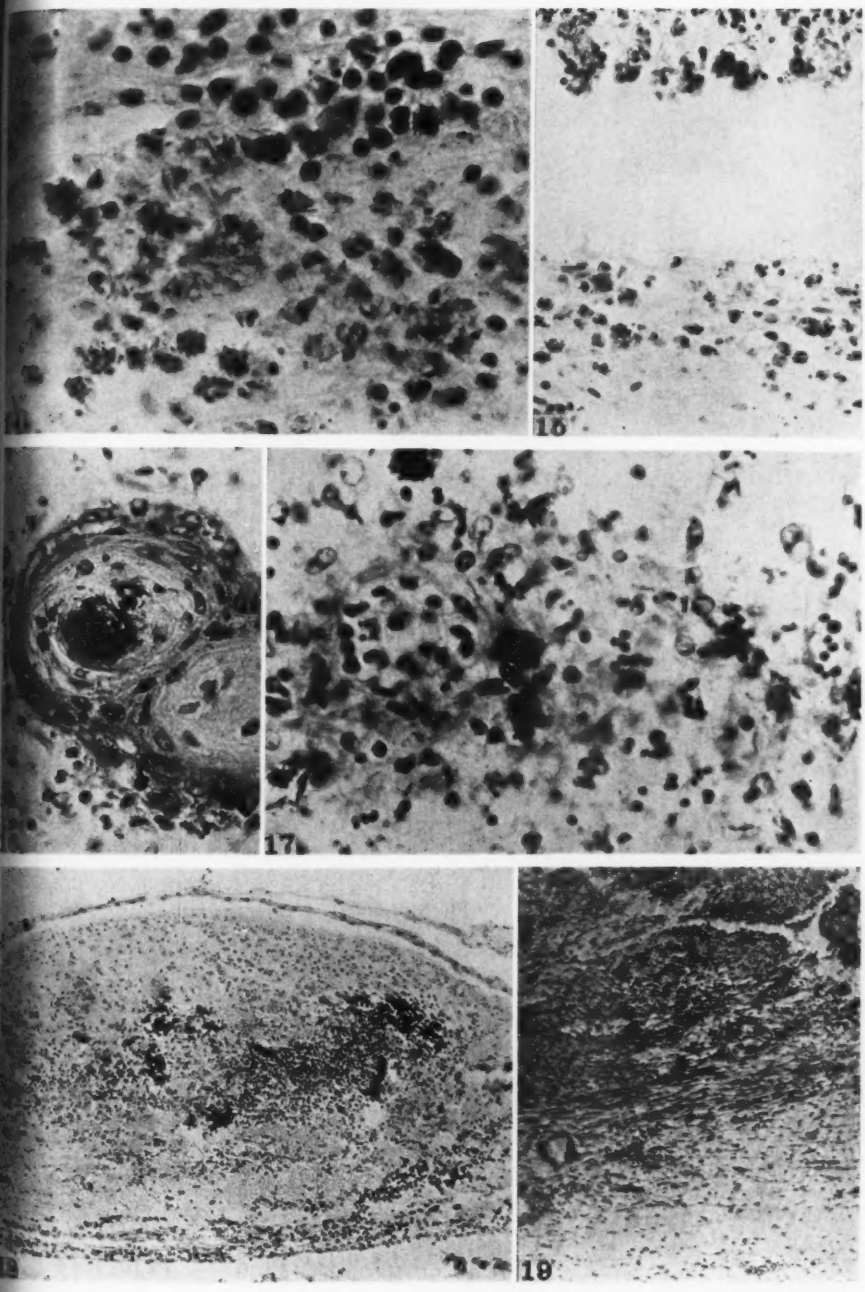
FIG. 15. Section through membrane showing separation of mesoderm from ectoderm. There is a foreign body response of macrophages at base of ectoderm and phagocytized bacilli in mesoderm. *Mycobacterium tuberculosis avium*. Acid-fast stain. × 420.

FIG. 16. Bacilli of *Mycobacterium marinum* in core of ectodermal "pearl." Acid-fast stain. × 525.

FIG. 17. Mesodermal response to *Mycobacterium marinum*, showing clumps and isolated bacilli and reaction around capillary. Acid-fast stain. × 700.

FIG. 18. Rapid invasion of mesoderm by *Mycobacterium marinum* on fourth serial passage. Acid-fast stain. × 130.

FIG. 19. Membranal response to inoculation with *Mycobacterium thamnopheos*. Giant cells and fibroblasts may be seen. Acid-fast stain. × 80.



Moore Mycobacteria in Chorioallantoic Membrane

PLATE 125

FIG. 20. Section through mesoderm, showing giant cells and increased number of capillaries. *Mycobacterium thamnopheos*. Acid-fast stain. × 360.

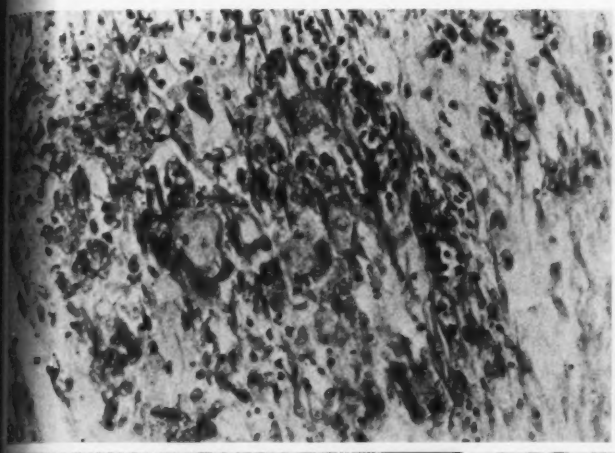
FIG. 21. Fused giant cells (tubercle) produced by *Mycobacterium thamnopheos*. Acid-fast stain. × 365.

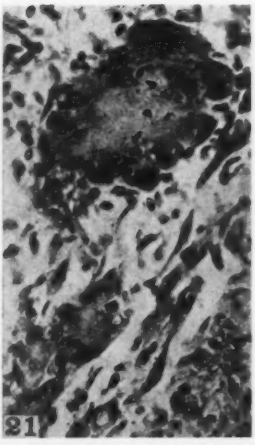
FIG. 22. Section through membrane inoculated with *Mycobacterium leprae muris*. There is ectodermal reaction and invasion of mesoderm by bacilli. Acid-fast stain. × 120.

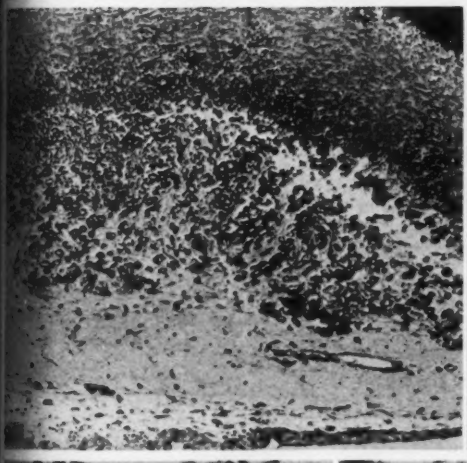
FIG. 23. Intracellular bacilli of *Mycobacterium leprae muris* in lepra or "foam" cells and in ectodermal extension. Acid-fast stain. × 445.

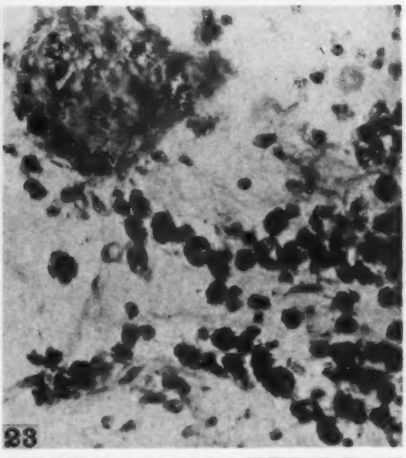
FIG. 24. Lepra or "foam" cells. Hematoxylin and eosin stain. × 500.

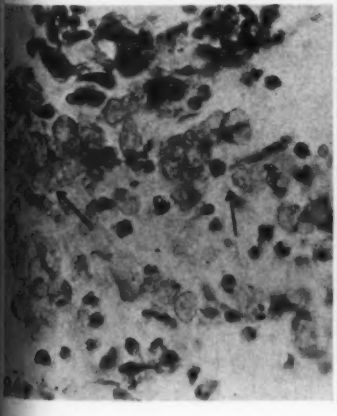
FIG. 25. Intracellular lepra bacilli. Acid-fast stain. × 840.

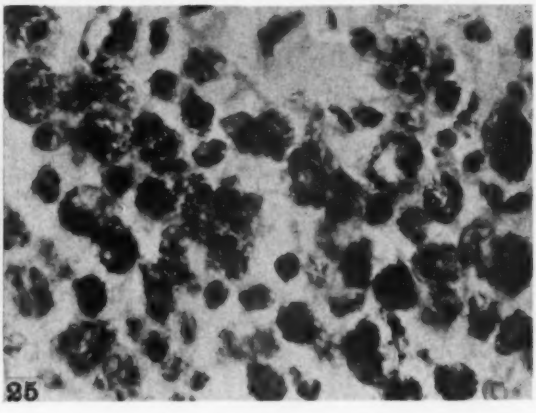












Moore Mycobacteria in Chorioallantoic Membrane

SENILE INVOLUTION OF THE THYROID GLAND *

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INTRODUCTION

It is the purpose of the present work to describe the changes occurring in the thyroid gland as a result of the aging process. Studies on the histology of the thyroid in senility are not wholly lacking in the literature, but both the observations and the interpretations drawn from them frequently are at variance and at present give anything but a clear-cut picture. This may be due, at least partly, to the fact that the human gland most frequently has been the object of study; and it is impossible with such material to eliminate factors of malnutrition, race and environment, and pathological condition. The present study confines itself to one experimental animal, the mouse, and chiefly to members of one pedigreed strain of that animal.

LITERATURE

In 1933 Dogliotti and Nizzi-Nuti [1] studied the thyroid glands of 53 individuals, 9 of whom were 70 years of age or older. On the histological side, they found reduction in the size of follicles with a corresponding decrease in amount of colloid, and an increase in the volume of the epithelial cells, with an increase in their content of granules. From these findings they were led to believe that there is in old age actually an increased degree of activity of the thyroid gland.

Again, in 1935, they [2] reported a study of the thyroids of 50 aged subjects, upholding their earlier conclusions. Speaking of the findings of a very early investigator on the subject (Clerc,[3] 1912), they said: "Alterations of regressive or degenerative type, such as described by Clerc in advanced age, have been observed by us at any period of life, when the cause of death was a serious infectious or exhausting disease." (Clerc had listed as characteristic senile changes: degeneration of epithelial cells, with fat and pigment appearing in them, a "concentration" or solidification of the colloid, and a more or less noticeable increase in connective tissue.)

Hinton [4] studied thyroids of 80 individuals varying from newborn to 89 years of age, dying from accidental causes. In his group there were only 2 over 60 years of age and the histological picture was quite variable. He stated: "So far we have been unable to draw conclusions as to what is normal thyroid tissue for the different ages." Rice,[5] on

* Received for publication, November 18, 1941.

the other hand, believed that definite criteria for normality at different ages can be established. Garau [6] found evidence of increased activity in old age in the thiner colloid in numerous follicles with high epithelial cells, as such thin colloid, according to his concept, is colloid recently formed. This increased activity in places he apparently considers as compensatory to the degeneration of follicles elsewhere, which are "smothered" or submerged by the proliferating connective tissue.

Nolan [7] reported on a very extensive series of autopsies (725 cases). He found in this series that as age increased the number of "normal" glands decreased, and he said: "Fibrosis is relatively more common with increase in age." His tables, however, subdivided his cases on the basis of pathological condition without giving the age of the individuals. Hertzler [8] found in old age an increase in the basophilic property of the colloid, some abortive attempts of follicles to reproduce themselves and changes involving the connective tissue. In general he regarded the changes seen as frankly regressive. Ghigi and Reggiani,[9] in a study on constitutional types, age and the thyroid, found in older persons a tendency to a stabilized condition of the thyroid, numerous larger follicles with "colloide statica" and a lining of squamous epithelium. They found also an increase in interfollicular masses of epithelial cells.

In one of the few studies on age changes in the thyroid in animals other than man, Lowe,[10] working with sheep, came to a similar conclusion. The condition of the secretion in which it appears solid and probably more or less "static" she designated as the "colloid condition." She found this to be the ordinary condition in her older animals, together with, in some, a thickening of the interfollicular connective tissue. Her "older animals," however, varied from "1 year 9 months, to 4 years and over."

An investigation which approached the problem of senile changes in the thyroid from a different aspect is that of Vogeler.[11] He used as a criterion for the activity of the thyroid glands the minimum amount of dried, powdered gland which would induce transformation of tadpoles. By this method he found a slight increase in activity of the thyroid in old men, as compared to those of middle age, and in old women he found no apparent decrease in its activity.

One recent paper (Smith and Starkey [12]) has dealt with age changes in the thyroid gland of the normal mouse, using large numbers of animals, ranging from birth to 250 days of age. Although their oldest animals were chronologically younger than our "middle-aged" animals, they found large areas of cystic degeneration in the glands of that group.

Materials and Methods

Twenty animals were used in this investigation, the majority of which were pedigreed black mice of the stock C57, purchased from the Jackson Memorial Laboratory at Bar Harbor, Maine. These animals can be divided conveniently into three groups: the young, the middle-aged and the senile.

In the young group there were 5 animals: 4 white mice, 2 females and 2 males, each 50 days of age, from a local laboratory, and 1 C57 black female mouse, 41 days of age, from the Jackson Memorial Laboratory.

The middle-aged group included 6 animals: males of 362 and 381 days, and females of 312 and (3) 381 days each.

The senile group included 9 animals: males of 706 and 719 days, and females of 586, 595, (2) 596, 693, 696 and 699 days.

The animals were kept in our laboratory for several days after arrival, receiving adequate food and water supply during this time. They were then killed by rapid etherization and the thyroid glands were removed, together with the larynx and the upper portion of the trachea, in order to disturb the structure of the gland as little as possible. The fixative employed was a 4 per cent solution of formaldehyde. Lowe,[10] after many trials, found this to be the most satisfactory fixative for the colloid, and we have seen practically no shrinkage attributable to it.

The tissues were then washed, dehydrated, infiltrated and embedded in paraffin. Frontal sections of the entire block of tissue were then cut at 8 to 10 μ, and the sections mounted and stained with hematoxylin and eosin. Large numbers of sections and various portions of the glands of each animal were observed. Both qualitative and quantitative studies were pursued. Numerous sections were observed and 500 or more follicles from each gland were given special study.

Observations

Sections of the thyroid glands from members of each of the three groups designated as young, middle-aged and senile displayed characteristics even under the low-power ($\times$ 100) which enabled one to distinguish them without difficulty.

In the thyroids of the young animals the follicles presented a very uniform appearance, both as to size and structure. Most of the follicles ranged from 30 to 50 μ in internal diameter; the maximum diameter being 85 μ. All of them were lined with cuboidal epithelial cells with spheroidal, vesicular nuclei. These cells possessed a compact, rather homogeneous, and readily stainable cytoplasm, with no vacuolation. The interstitial material was fairly abundant and consisted chiefly of immature adipose tissue. It tended to separate groups of follicles rather

than individual follicles from one another. The follicles, almost without exception, contained large amounts of colloid of a rather lightly acidophilic nature, many of them being completely filled and many having a mass of homogeneous colloid in the center, with a space between the colloid and the cells. The colloid, at least in this fixed material, was seen to be of an almost homogeneous but very finely vacuolated structure, the entire body of the colloid being of this type.

The thyroids of the animals in the middle-aged group were different in many respects from those of the young animals. In the middle-aged animals the follicles occupied almost the entire bulk of the gland, with only small amounts of interfollicular material showing between them. The follicles were on an average much larger than those of the young animals, although the range in size was considerable. The maximum internal diameter of these follicles was 175 μ. The colloid here was considerably more eosinophilic than in the younger animals. It filled the follicles and seemed even to distend them, most of the larger follicles being lined with a very low cuboidal or definitely squamous type of epithelium. The colloid in these follicles was homogeneous. The cytoplasm of the epithelial cells was well stained, the nuclei of the definitely cuboidal cells were vesicular and those of the squamous cells pyknotic. The interstitial material, only very small amounts of which were present, was similar to that in the young mice.

The appearance of the thyroid glands of all of the senile animals was very different from that found in either the young or the middle-aged animals. The two most conspicuous features were, first, the great increase in definitely fibrous connective tissue and, second, the loss of a large proportion or of all of the stainable colloid from very large numbers of the follicles. These changes were definitely more marked, in our series of animals, in the females, although the male animals showed them in varying degree. The follicles presented a remarkably varied aspect, even within the same field. There were many apparently empty follicles; others showed a few shreds of colloid or small clumps of granular material; still others presented considerable amounts of colloid in the form of concentric rings, the "layers" of which differed sharply in staining capacity. In this latter group the central mass, conforming more or less to the shape of the whole follicle, was at times of a reddish purple tinge, evidently possessing some basophilic quality as well as its eosinophilic property. The outer layers stained much less deeply and appeared less solid, frequently showing a minute vacuolation (Fig. 3). In some places the central mass was split, reminding one of fissures in hardened jelly, while this was never true of the more lightly stained outer layers. The empty follicles, and also those with only small amounts of colloid remaining, were frequently distorted, elongated or

angular, rather than presenting the round or oval forms which were the only shapes found in the younger age-groups. In addition to the irregularly shaped follicles, there were numerous groups of epithelial cells surrounded on all sides, as made out by study of serial sections, by dense fibrous connective tissue. These we believed to be the remains of follicles. Certainly, in a given volume of gland there were far fewer follicles in the senile than in the middle-aged mice. While there usually was no sign of a lumen in these interacinar epithelial cell groups, the arrangement of the cells was sometimes very suggestive of "smothered" follicles.

In addition to the great variety in shape and in colloid content of the follicles in old age, there were such differences in size that minute follicles, 20 to 30 μ in internal diameter, were seen beside enormously distended follicles, reaching a diameter of 400 to 500 μ. In such distended follicles the epithelium consisted of greatly flattened cells, and where the thin walls of these large follicles came together, rupture had occurred in many instances, so that bilocular or trilocular follicles were distinguished (Fig. 6). The epithelial cells of the senile follicles showed profound changes as compared to those of younger animals. The higher cuboidal cells showed nuclei still vesicular but of less intense staining qualities than formerly and irregular or "shrunken" in shape. The cytoplasm was very pale and highly vacuolated, with the more stainable portion forming a coarse meshwork of strands or gathered near the base of the cell (Fig. 4). The nuclei of the squamous epithelial cells were pyknotic.

All of the interfollicular substance was fibrous connective tissue with nuclei of connective tissue cells lying with their axes parallel to the direction of the fibers.

With high magnification, it was possible to see in many of those follicles which under low-power seemed entirely empty, a tenuous coagulum of material occupying part or all of the follicle and probably representing, it seemed to us, the remains of a thin fluid present in the follicle during life. Such an appearance was seen also about the periphery of the solid, fissured masses in the center of many of the follicles.

Quantitative data on the thyroids were obtained by a study of 500 or more sections of follicles in each animal. Table I gives a comparison between some characteristics of the follicles in the 20 animals studied. This comparison, it should be noted, is actually between sections of follicles rather than whole follicles, although the term "follicle" is used for convenience in the table. However, the classification of 500 such sections should give a fairly accurate idea of the general histological picture of the gland. In classifying it was customary to use every other section on the slide.

Discussion

We feel that the present study shows a definitely regressive process, a true histological involution, of the thyroid gland in senility. This process appears similar to involution as seen in other glands, for instance, the mammary gland. There is a great increase in the amount of connective tissue; such an increase that the follicles frequently appear like islands in this proliferating fibrous material.

Table I

Classification of 500 or More Sections of Follicles in Each of 20 Mice According to the Amount and Type of Colloid Present

Animal, age in days and sex	Total no. of follicles classified*	Full†		Partially full‡		Partially full§		Empty	
		No.	Percentage of total no. of follicles	No.	Percentage of total no. of follicles	No.	Percentage of total no. of follicles	No.	Percentage of total no. of follicles
41 ♀	543	528	97.2	0	0	15	2.8	0	0
50 ♀	647	269	41.6	0	0	374	57.8	4	0.6
50 ♀	500	91	18.2	0	0	404	88.8	5	1.0
50 ♂	529	369	69.8	1	0.2	157	29.7	2	0.4
50 ♂	519	251	48.4	1	0.2	260	50.1	7	1.3
312 ♀	551	545	99.0	2	0.3	4	0.7	0	0
362 ♂	556	544	97.7	2	0.4	8	1.4	2	1.4
381 ♂	517	492	95.2	0	0	24	4.6	1	0.2
381 ♀	585	507	86.8	2	0.3	72	12.3	4	0.7
381 ♀	526	305	58.0	2	0.4	190	36.2	29	5.5
381 ♀	529	310	58.6	17	3.2	198	37.4	4	0.8
719 ♂	503	330	65.6	14	2.8	152	30.2	7	1.4
706 ♂	506	190	37.5	12	2.4	295	58.3	9	1.8
586 ♀	520	18	3.5	6	1.2	434	83.5	62	11.9
595 ♀	559	8	1.1	6	1.1	376	63.5	169	30.2
596 ♀	522	18	3.4	10	1.9	366	70.1	138	26.4
596 ♀	505	11	2.2	15	3.0	335	66.3	144	28.5
693 ♀	527	36	5.8	10	1.9	315	59.8	166	31.5
696 ♀	585	19	3.2	2	0.3	235	40.2	329	56.2
699 ♀	537	85	16.4	6	1.2	235	45.5	191	36.9

* All follicles are here classified as they appear under low power (× 100).

† Those follicles in which the stainable colloid completely fills the lumen.

‡ Those follicles in which the colloid completely fills the lumen but in which it is in the form of two or more rings, of different staining capacity (Fig. 3).

§ Those follicles in which an apparently empty space, large or small, surrounds the colloid which may itself vary from fairly abundant to very sparse in amount.

Dogliotti and Nizzi-Nuti [1,2] found no noteworthy increase in such connective tissue in thyroids of senile human beings. Others, however, have suggested it. Clerc [3] had stated that an increase in connective tissue occurs in old age. Rice [5] found an increase in interacinar connective tissue and also of interacinar groups of epithelial cells. Hertzler [6] found "changes involving the connective tissues"; and Garau [6] found varying degrees of increase in this element. Nolan,[7] on the other hand, concluded that it was not possible from his studies to say what is normal thyroid tissue for different ages.

Dogliotti and Nizzi-Nuti [1,2] believed that a compensatory hyperfunction of the thyroid occurs in old age, basing this opinion partly on what they thought to be epithelial proliferation and, more largely, on the

sparse content of colloid in the follicles in many of their senile specimens. Garau [6] thought that evidence of increased activity might be noted in some epithelial hypertrophy and in the presence in numerous follicles of a very thin colloid, supposedly of "recent" formation.

We believe that the small amount of stainable colloid seen in the old animals means rather that the gland has gradually been depleted of this substance. The very thin, fluid colloid seen then to fill or partially fill many follicles would represent, in all likelihood, colloid in process of absorption through the epithelial cells to the blood vessels rather than recently formed secretion. The presence of the very solid-appearing and frequently split colloid in some follicles would tend to support this view of a more static condition of the gland, with secretion now overbalanced by reabsorption, but even that proceeding less rapidly, perhaps, than in the younger animals.

In regard to the interacinar collections of epithelial cells, we agree with the opinion of Garau [6] that these represent degenerate follicles, robbed of their stored secretion, no longer capable of secretion and forced into mere clumps of cells by the encroaching connective tissue.

The enormous distension of some of the follicles in the senile animals, with the extreme flattening of their epithelial cells, and in some instances the rupture of one or more follicles into one another, is an interesting phenomenon. It may be similar to the phenomenon which Smith and Starkey [12] described as "cystic degeneration" in their older animals. While their "old" animals were less than half the age of ours, they were using a different type of mice—albino mice of their "Hygienic Strain"; and it seems possible that the life-span may vary in different strains of mice. Their observations on the histological character of the thyroid in young and middle-aged animals agree well with ours in regard to the amounts of colloid and of interfollicular material and to the size of the follicles and nature of the epithelial cells lining them.

While senile changes of the same general type occur in both sexes, these changes were far more marked among the females in the animals which we have studied. Further work is needed to show whether this apparent sex difference is a constant one.

It has not been the purpose of the present study to attempt to solve the problem of the mechanism of thyroid secretion, but some of the findings here reported seem so pertinent to that subject that we shall discuss them briefly.

A question frequently recurring in the literature is that of the reality in life and the significance of the vacuoles in the colloid. Some recent papers by authors using very modern methods of investigation tend either to deny the existence or to minimize the importance of such

vacuoles. Gersh and Caspersson [13] analyzed the colloid of thyroid follicles of guinea pigs for total protein and iodine content, using the absorption of ultraviolet light by this substance to determine its chemical nature in areas as small as 0.5 square μ. They stated: "No evidence whatever could be uncovered indicating significant unhomogeneity of the colloid." This refers to the colloid within any given follicle. From follicle to follicle, the colloid differed considerably in chemical constitution.

Williams,[14] in studies on living follicles, has observed a definite cycle of changes in the size of the lumen and the thickness of the wall. He, too, was inclined to minimize the functional significance of apparent lack of homogeneity of the colloid. He said: "That the colloid may escape from holes in the wall or by definite channels in or between the cells is not indicated by these studies." It would seem that the elimination of these alternatives would leave some type of digestion or liquefaction and absorption of the colloid, through the epithelial cells, as digested foods are carried through the cells of the intestinal tract, as a likely method. It is rather interesting in this regard that the active principle of the thyroid gland is not destroyed by digestive enzymes, as are the hormones of many other glands. Embryologically, of course, the thyroid gland is a derivative of the upper part of the digestive tract, along the walls of which many glands with digestive secretions are developed. In this regard, a new concept developed by Leach [15] which suggests that the endostyle of ammocoetes has at least two functions: first, the formation of a secretion which mixes with the food, and second, the absorption of certain elements dissolved in the water circulated by its ciliated cells, is of considerable interest, as suggesting an original dual rôle for the nonciliated cells of the thyroid in secretion and absorption. This idea of secretion with later digestion and reabsorption is not entirely new. Thus Gersh and Caspersson [13] said: "The colloid in the lumen may be digested by the action of enzymes secreted there by the cells."

De Robertis,[16] using young and "normal adult rats," also stressed the homogeneity of the intrafollicular colloid, as seen after preparation of the tissue by the freezing-drying method. He believed that chromophobic vacuoles, when seen, are usually artefacts of fixation. Colloid release, according to this author, is brought about by transcellular transport of the intrafollicular colloid and also, when the release is very active, by inclusion of colloid by the thyroid cells. The presence of such demonstrable colloid droplets in the cells, however, could hardly be said completely to contradict the idea of alteration of the colloid before its reabsorption by these cells. Certainly many of the

intracellular droplets demonstrated by De Robertis represent colloid in process of being secreted.

The present study on the nature of the colloid at different stages in the life cycle shows a striking lack of homogeneity in this substance in old age, a period in which the amount is greatly diminished, without new secretion to balance the loss. It is at this period that we would expect, then, to see visible evidence of the changes in the colloid prior to reabsorption. Our observations and figures demonstrate that wherever there is lack of homogeneity in the colloid, the thinner, minutely vacuolated parts are nearer to the periphery, while the more solid mass, which we might almost conceive as "melting down" into the fluid portion, lies in the center of the follicle. We readily admit that the chromophobic vacuoles seen by many earlier authors, after various types of fixation, may be artefacts, but the great lack of homogeneity of the colloid in the thyroids of senile mice could not be interpreted as artefactual.

Conclusions

1. A study of the thyroid gland in young, middle-aged and senile mice shows marked qualitative and quantitative differences. These differences are greater between middle-aged and senile animals.

2. The thyroids of young animals show relatively small follicles, cuboidal epithelial cells and an almost homogeneous colloid present in large, though varying amounts. There is a fairly large amount of interfollicular material, consisting chiefly of immature adipose tissue.

3. In middle-aged animals the follicles are larger on the average, with low cuboidal or squamous epithelium, and a homogeneous colloid which fills the lumen. There is very little interfollicular connective tissue.

4. In senility there is an enormous increase in the fibrous connective tissue between the follicles. There is an atrophy of some follicles, a hyperdistension of others. The colloid presents an extremely varied picture. Many follicles lack stainable colloid entirely. Others contain only traces, sometimes in clumped granular form. Others show definite stratification, with solid, fissured colloid in the center and thinner, finely vacuolated colloid at the periphery and next to the epithelial cells.

5. The thyroid gland of the mouse undergoes a definite histological involution in old age.

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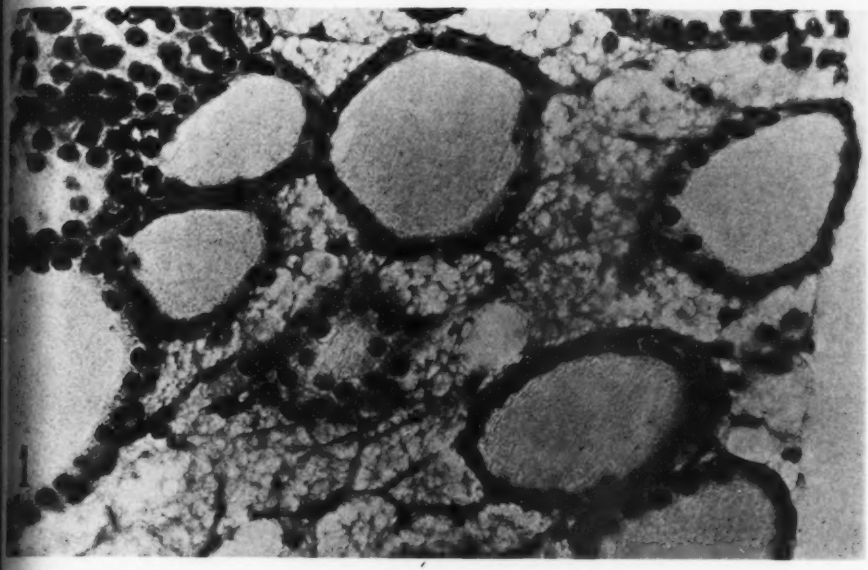
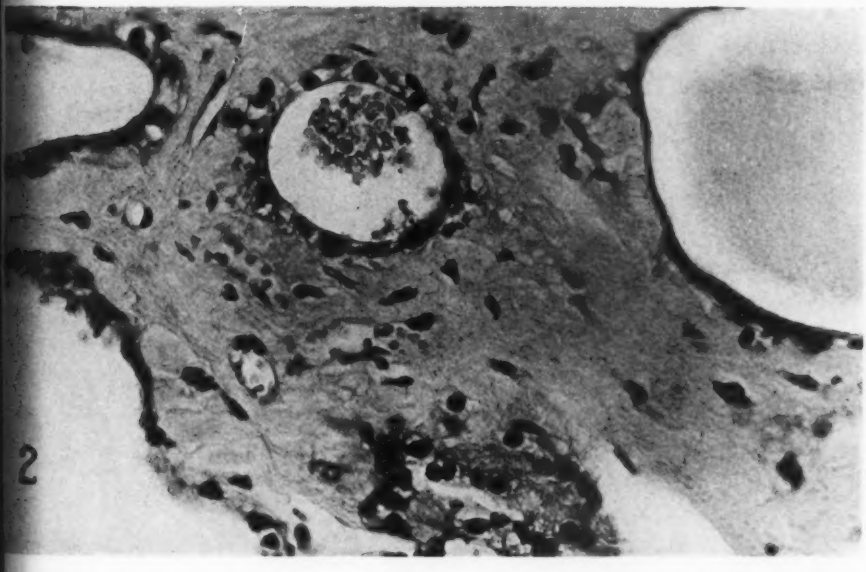
DESCRIPTION OF PLATES

PLATE 126

FIG. 1. Thyroid gland of a 41-day-old female mouse. The follicles are filled with a finely vacuolated colloid. The epithelial cells are fairly high cuboidal, and the interstitial material, present in considerable amount, but very loosely meshed, consists of immature adipose tissue. × 470.

FIG. 2. Thyroid gland of a senile mouse, a 596-day-old female. There is a large amount of dense fibrous connective tissue. The follicles are, for the most part, only partially filled with stainable colloid or are entirely empty. The colloid is variable in appearance. The mass of epithelial cells in the lower central portion of the field is probably a degenerate follicle. × 470.



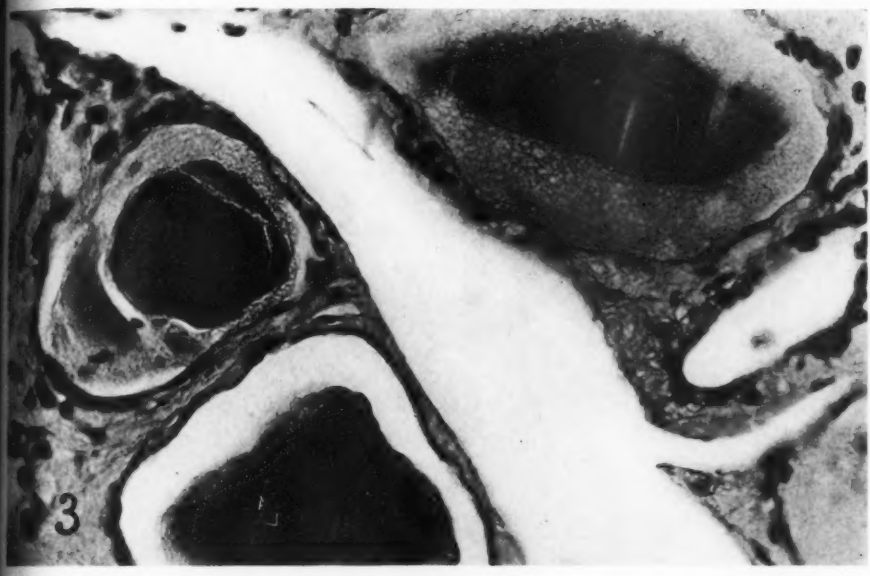


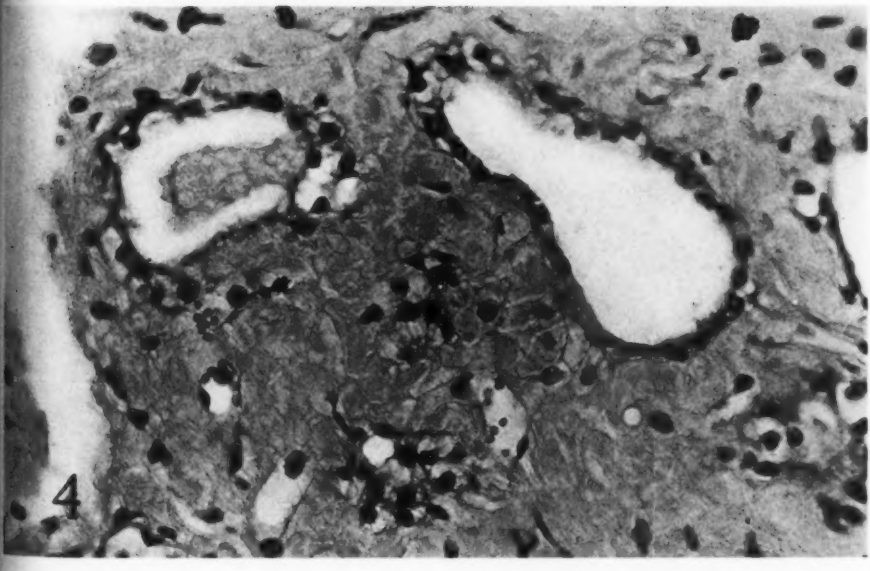
Andrew and Andrew Senile Involution of the Thyroid Gland

PLATE 127

FIG. 3. Thyroid gland of a senile mouse, a 693-day-old female. The follicles in the field show the very solid-appearing central colloid and the thinner, vacuolated colloid which is apparently a step toward the absorption of the material. × 470.

FIG. 4. Thyroid gland of a senile mouse, a 595-day-old female. The larger of the two follicles shown has an elongated, irregular shape and is empty of stainable colloid. The smaller follicle, on the left, shows a small amount of thin, vacuolated colloid still present. There is a large amount of interfollicular connective tissue and the epithelial cells show degenerative changes. × 470.



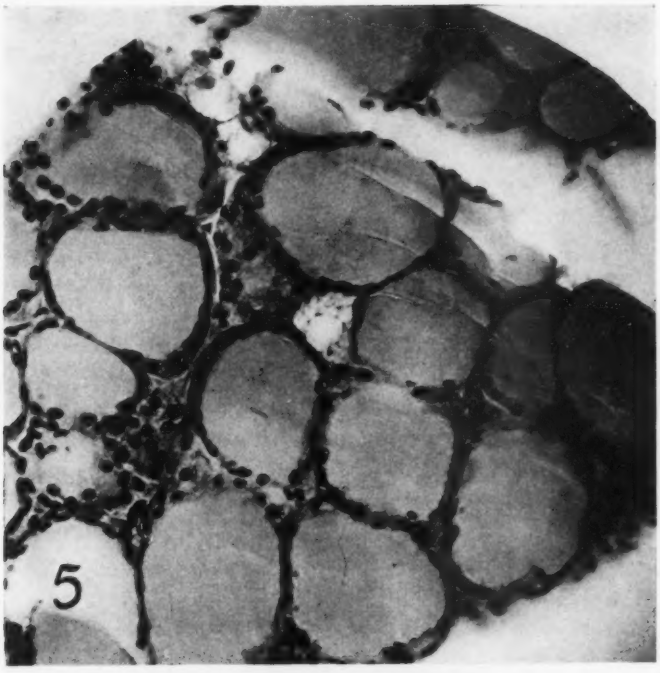


Andrew and Andrew Senile Involution of the Thyroid Gland

PLATE 128

FIG. 5. Typical field from the thyroid gland of a mouse of middle age, a 312-day-old female. The follicles are filled with homogeneous colloid, with low cuboidal epithelial cells lining the lumen. × 235.

FIG. 6. A group of follicles from a senile mouse, a 596-day-old female. Here rupture of the greatly stretched epithelium has occurred in several places and a group of communicating follicles has been formed. × 235.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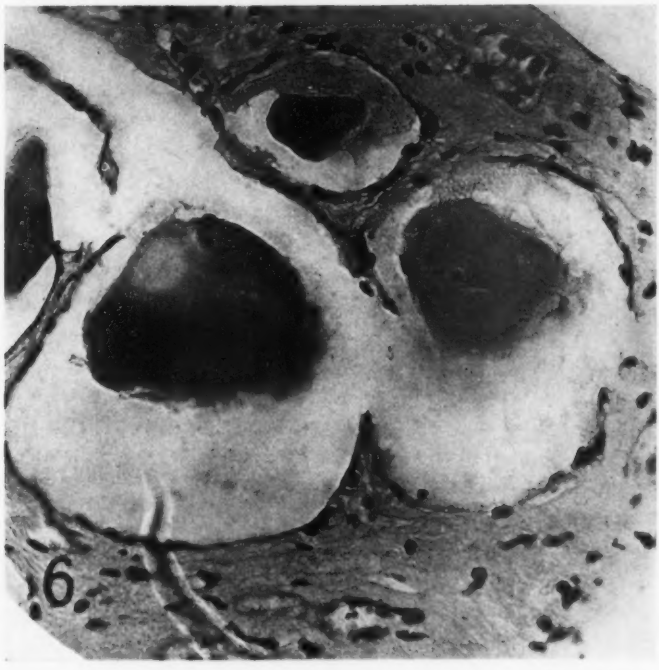
6

PERIPHERAL NERVES IN CHRONIC ATROPHIC ARTHRITIS*

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Occasionally in diseases of unknown etiology, organic pathologic changes are found which do not manifest themselves clinically. Recently the muscles and valves of the heart in chronic atrophic arthritis have been examined and noteworthy changes have been described.[1] At the suggestion of one of us an investigation of the central and peripheral nervous system in cases of chronic atrophic arthritis was undertaken. In the 2½ years of our investigation, previously undescribed pathologic changes have been found. This communication reports these findings.

At the beginning of this investigation of the involvement of the nervous system in chronic atrophic arthritis, only brains and spinal cords were examined. No specific or characteristic lesions were found. Later, in addition to the central nervous system, peripheral nerves and the sympathetic chain were studied. Fourteen cases diagnosed as chronic atrophic arthritis came to autopsy. A study of the clinical features and the pathologic findings of 3 of the cases revealed that the clinical diagnosis was questionable. Thus 11 cases remained in which the clinical and the pathologic diagnoses were established beyond doubt. Roentgenograms confirmed the diagnosis in each case and showed narrowing of the joint spaces, sharply edged margins of the joints, destruction of subchondral bone, atrophy of epiphyseal bone and occasional swellings of periarticular soft parts. The central nervous system was examined in each of these 11 cases. In 5, the peripheral nerves also were examined.

The nerves were fixed in a 4 per cent solution of formaldehyde. The following stains were used in histologic examination: hematoxylin and eosin, van Gieson, Giemsa (Lillie), cresyl-violet, Bodian's stain for axis cylinders, Tibor Pap's stain for reticulin,† all on paraffin-embedded material; Spielmeyer's myelin sheath stain, and fat stain (scarlet red) on frozen sections. Frozen sections were usually made; the remaining tissue was brought back into formaldehyde and then cut into smaller blocks which were embedded in paraffin and stained. Forty to 70 paraffin blocks of peripheral nerves from each case were examined.

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† Pap, Tibor. Eine neue Methode zur Imprägnation des Retikulums. *Zentralbl. f. allg. Path. u. path. Anat.*, 1929–30, 47, 116–117.

In some instances threads were attached to each end of the nerve. The threads were inserted into small glass tubes and the nerves then submerged in a jar containing a 4 per cent solution of formaldehyde for fixation in a perfectly straight direction.

A clinical and pathologic summary of each of the five cases in which the peripheral nerves were examined follows:

Reports of Cases

Case 1

A. S. was a white female, 55 years of age, with arthritis of 18 years' duration. This patient showed involvement of most of her joints with stiffness and finally ankylosis; parchmentlike, scaly, and in places shiny and atrophic skin, and some rigidity of the spine in all segments. The patient was subjected to manipulative procedure under anesthesia and in 1 hour and 20 minutes developed deep breathing and cyanosis. Death occurred 2 hours after operation.

Pathologic Examination

The following pathologic diagnoses were made: chronic atrophic arthritis, chronic rheumatic heart disease with mitral valvulitis and pericardial adhesions, fat emboli of lungs and kidneys, chronic passive hyperemia of viscera, recent small hemorrhages in the floor of the fourth ventricle of the brain and fat emboli without hemorrhages in the brain and the spinal cord.

Peripheral Nerves. Femoral and tibial nerves from both sides were examined. In cross sections as well as in longitudinal sections stained with hematoxylin and eosin, small nodules of dark color were seen with the naked eye. They were 0.1 to 0.3 cm. in longitudinal diameter and shorter in the transverse diameter, so that a rather regular oval shape resulted. There were usually two to three such nodules in one slide. However, slides of adjacent sections were frequently devoid of these nodules. The nodules were located almost exclusively in the perineurium, never in the endoneurium and rarely in the epineurium. Figure 1 is a characteristic picture of this location between two sheets of the perineurium, representing a cross section through the peripheral nerve. A histologic analysis of these nodules showed that they consisted of three different zones: a central acellular zone of homogeneous coagulated material occasionally containing nuclear débris; an intermediate zone consisting of large, irregularly shaped, hypochromatic nuclei with more or less definite nucleoli, and a peripheral outer zone containing numerous lymphocytes and plasma cells (Figs. 2 and 3). A sharp demarcation of the lymphocytic peripheral zone from the surrounding normal peripheral tissue was always present. The homogeneous central masses did not take the typical blue-black color of fibrin in Weigert's differential stain. Other stains such as phosphotungstic acid hema-

toxylin or Mallory's aniline blue as well as van Gieson's stain revealed collagenous strands between the homogeneous masses, but likewise no typical fibrin reactions. Reticulin stain revealed a number of fibrils between the masses. Giant cells or multinucleated isolated cells were not seen. The separation into three zones was not sharp. There was usually a more or less indistinct merging, particularly between the intermediate and peripheral zones. The central homogeneous acellular zone was sharply demarcated, however, from the intermediate zone.

Deviations from the regular three-zonal arrangement were often observed. For example, there sometimes was no acellular central zone; only the intermediate epithelioid cell zone and the peripheral lymphocytic ring. In other instances the inner zone contained a central blood vessel which in its outer walls, particularly in the adventitial parts, was filled with the usual amorphous and acellular material. The intermediate zone was then smaller than is usually seen or was represented by a few epithelioid cells. A cross section through the nerve showed the typical perineural location, a thickened arteriolar wall, and acellular masses in a small part of the vessel wall located in the adventitia and also more peripherally (Fig. 4). There were particles of nuclear débris only in the part of the vascular wall near the acellular zone. A slight deviation from the usual appearance showed the epithelioid intermediate zone not in the center but in a more peripheral location. A loose network, but not a typical lattice of connective tissue, was present at the periphery of the acellular central masses. The nerve fibers themselves, as shown in Spielmeyer's stain for myelin sheaths, in Bodian's stain for axis cylinders and in sections stained for neutral fat (scarlet red), were normal throughout. In this case 65 different single nodules were examined from 30 paraffin blocks. Sections of 16 of these blocks showed nodules; sections of 14 blocks were negative. This gave an average of 4 nodules in 1 positive section.

Case 2

Z. F. was a white female, 63 years old, having atrophic arthritis of 16 years' duration, with involvement of hands, knees and toes; right upper lobe pneumonia, and hypertension.

Pathologic Examination

The following pathologic diagnoses were made: chronic atrophic arthritis, lobar pneumonia, marked generalized arteriosclerosis, severe coronary sclerosis, nephrosclerosis and multiple small hemorrhages in both thalami and occipital lobes due to hyalinosis of the walls of the arterioles.

Peripheral Nerves. Sixty-five different paraffin blocks were cut from four nerves (sciatic, femoral, tibial, axillary). Inflammatory nodules

were seen in only five blocks. Again, the characteristics were the location in the perineurium and the sharp demarcation from the surrounding tissue. These nodules, however, showed only massed lymphocytes and plasma cells. The central acellular zone and the intermediate epithelioid zone were not seen. Occasionally a few epithelioid cells were observed as a rudimentary feature of an intermediate zone. No changes in the nerve fibers were seen with fat, myelin sheath, or axis cylinder stains.

Case 3

M. R., a white female, 73 years of age, had atrophic arthritis of 4 years' duration, with involvement of the elbows and of the metacarpal-phalangeal joints; tenderness of the left shoulder, left wrist, knees and ankles; cellulitis of the right elbow with septicemia due to hemolytic streptococci.

Pathologic Examination

The following pathologic diagnoses were made: atrophic arthritis, septicemia, septic spleen and liver, coronary sclerosis and occlusion with myocardial infarct.

Peripheral Nerves. Forty-two different paraffin blocks were cut from two nerves (femoral, tibial). Only two nodules were found of the pure lymphocytic type described in case no. 2. Here also the perineural location and the nodular character were noted.

Case 4

M. D. was a white female, 61 years old, with the duration of her atrophic arthritis not known. She showed the following: typical fusiform swelling of fingers of right hand; arthritis of both wrists and elbows; bilateral ulnar deviation; Heberden's nodes on distal phalangeal joints; hypertensive heart disease; enlargement of heart to left; blowing apical and aortic systolic murmurs; blood pressure, 170/138 mm. of Hg.

Pathologic Examination

The following pathologic diagnoses were made: atrophic arthritis, hypertrophic arthritis, coronary thrombosis of anterior descending branch, recent myocardial infarct, aortic and coronary sclerosis, thickened mitral valve, arteriolar nephrosclerosis, healed tuberculosis of lung, chronic salpingitis and hyperemia of the leptomeninges. No focal lesion was found in the brain.

Peripheral Nerves. Thirty-eight different blocks were cut from two nerves (femoral, axillary). Twelve single nodules were found; three in the same slide from one block, the others in single slides from nine different blocks. In these nodules there were two distinct zones, the intermediate and the peripheral lymphocytic zone. The central acellular zone was absent. The nodules were oval or spherical. They were, with the exception of one which was epineural, situated between perineural folds, as is seen in Figure 5. The nodules were sharply demar-

cated and they contained more collagenous bundles in the intermediate zone than were seen in other cases. There was a diffuse thickening of the perineural coverings of nerve bundles. One inflammatory nodule in epineural fat tissue was seen. A peculiar finding in this case was a perineuritic nodule encasing the total circumference of a small isolated nerve bundle. Here only lymphocytic infiltration was seen. The lymphocytes did not enter the endoneurium; they were not seen between the nerve fibers of this bundle. The intermediate zone was seen in a number of nodules. There was a zone of characteristic epithelioid cells, but there were numerous collagenous fiber bundles in this zone. Occasionally the intermediate epithelioid zone was more eccentrically located than usual. No nerve fiber changes were found in myelin sheath, axis cylinder, or fat stains.

Case 5

E. B., a white male, 49 years of age, had atrophic arthritis of 7 years' duration, with involvement of hands, shoulders, knees, ankles and hip; chronic iritis and secondary glaucoma, and chronic pyelitis.

Pathologic Examination

The following pathologic diagnoses were made: atrophic arthritis; septicemia due to hemolytic streptococcus with acute bacterial mitral and aortic valvulitis, and myocarditis; amyloidosis of liver, spleen, kidneys, adrenals, lymph nodes; chronic bilateral pyelonephritis; congenital atrophy of superior pole of left kidney with compensatory hypertrophy of right kidney. No focal lesion of the brain was found.

Peripheral Nerves. Forty-seven blocks were cut from 3 nerves (right and left femorals, right axillary). Twenty-two single nodules were found in 18 different blocks. Sections from 14 blocks showed only 1 nodule in each section. Here again, location, shape and demarcation were similar to those in previous cases. The nodules were located exclusively in the perineurium. They were oval or ellipsoid and sharply circumscribed. An acellular central zone was not present, but the intermediate and peripheral lymphocytic zones were seen. In this case epithelioid cells and lymphocytes were more intermingled; a distinct separation of the intermediate zone from the peripheral lymphocytic zone was in most instances not so clearly defined as in other cases.

Kahn and Kline tests of blood were negative in all cases.

Morphology of the Lesions

The findings in the peripheral nerves can be summarized as follows: The principal characteristic finding was a *nodular inflammatory* lesion. This lesion consisted of accumulations of lymphocytes and plasma cells

in the perineural nerve sheath or, less frequently, in the adjacent epineural tissues. The nodule cannot be seen grossly in the unstained specimen, but with hematoxylin and eosin and with other methods of staining, it was seen with the naked eye as a small round or oval nodule. Occasionally the nodule covered the entire surface of a small nerve bundle, uniformly infiltrating the perineurium and its outer regions without penetrating the nerve bundle itself and its endoneurium. The perineuritic nodules were often found in groups of three or four in the same section. They were scattered through a portion from 2 to 2.5 cm. of the longitudinal course of a peripheral nerve. Very often the adjacent blocks of the same nerve were completely free from these nodular lesions. There was no specific distribution of the nodules except their location in the perineurium.

The size of a single nodule varied. Serial sections through such a nodule in paraffin-embedded material showed an extension of a nodule through 20 to 25 sections of 7 μ each; that is, 140 to 175 μ (0.14 to 0.175 mm.). The maximum diameter observed was 0.2 mm. Assuming that the embedding process leads to a general shrinkage of the tissues by use of dehydrating agents and xylol, the original size of the nodule may be somewhat larger.

A more detailed histologic analysis of the nodule showed a complicated pattern consisting of two or three different layers. The first was a lymphocytic outer zone with rather numerous plasma cells in the most peripheral part; the second was a middle zone of polyhedral cells having large and irregular nuclei poor in chromatin, cells comparable to the epithelioid cells in gummatous or other granulomatous tissues; the third and innermost zone consisted of a pale and *acellular* center with homogeneously staining deposits in which occasionally small dark granules of nuclear detritus were embedded. Capillaries and other small vascular channels were found in all parts of the nodule, even in the innermost homogeneous part where well preserved vascular walls were seen. The endothelial cells of the vascular walls were normal or slightly swollen, and the adventitial spaces of the vessels were occasionally filled with the same homogeneous material which had spread without clear-cut limitation into the immediately adjacent structures. Various stains to identify the homogeneous deposits showed that this material did not give a definite fibrin reaction. It stained deep purple with hematoxylin and eosin and greenish with cresyl-violet. Reticular fibers ran through it. In some places true collagenous fibers were seen in outer portions of the deposits. In other nodules in the same section the homogeneous center was lacking, and only one or both of the distinct outer zones were found; *i.e.*, the peripheral lymphocytic zone

alone or combined with the inner polymorphous cell layer, with cells of the epithelioid cell type. The size of the three zones varied: the middle layer of epithelioid cells and the innermost homogeneous acellular central zone were usually smaller than the outer lymphocytic ring.

Of special interest are the so-called epithelioid cells. These were mesodermal cells, the nuclei of which resembled the nuclei of the perineural mesodermal elements. The nuclei were elongated, poor in chromatin and almost always irregularly shaped. As seen in cresyl-violet, methylene blue or Giemsa (Lillie) stains, epithelioid cells formed a symplasm. Clear-cut cell outlines were not demonstrable, but there was a faintly stained cytoplasm (Fig. 6). The origin of these epithelioid cells is obscure. Their derivation from the endothelial cells of the blood vessel walls or from other vascular mural cells is not probable for the reason that there is occasionally, in the innermost homogeneous zone, a blood vessel with its walls completely unchanged, and for the reason that the epithelioid cell layer is often not connected with peripheral zones of the vascular wall. There is a complete separation of the vascular cells from the epithelioid cells by the homogeneous acellular masses.

From serial sections it was seen that some of the apparently pure lymphocytic nodules had an epithelioid center. Homogeneous masses, however, were not regularly found even by serial sections. For this reason it was assumed that they represent a more transitory phase of the nodule. Since serial sections could not be made of every nodule, the statement that there was an epithelioid cell center in *each* nodule may be questioned. However, in eight out of ten serial sets, the epithelioid inner cell layer was found even when the sections made first showed only lymphocytic nodules.

The sharply circumscribed appearance of the nodules should be emphasized. The outer lymphocytic ring was sharply demarcated from the surrounding perineural tissue, which was entirely normal. Hence the use of the term "nodule" is justified.

In each of our five cases of chronic atrophic arthritis in which peripheral nerves could be examined, similar changes were found. Cases nos. 1, 4 and 5 showed well developed identical lesions; in cases nos. 2 and 3 the lesions were more rare and were not fully developed. There seems to be a relationship between the age of the patient and the severity of the lesion, for the younger patients showed more severe lesions than the older ones. In cases nos. 1, 4 and 5 the patients were 55, 61, and 49 years of age respectively; in cases nos. 2 and 3 the patients were 63 and 73 years old. Since the findings in the peripheral nerves were not foreseen and these lesions were not expected, no par-

ticular attention was given to a correlation between the degree of involvement of the various *joints* of the four extremities and the severity of lesions in the *peripheral* nerves. The nerves collected were those which could be removed during autopsy without too extensive additional cutaneous incisions (axillary, sciatic, tibial, femoral and sympathetic chain). Only nerve trunks were removed. The more peripheral portions close to the joints were not examined. Therefore, nothing can be said about the intensity of the peripheral nerve changes near the joints, or about the relationship between the severity of the regional joint lesions and the lesions in the peripheral nerves. The small number of nerves examined (five cases) does not permit us to say anything about the relationship between the *duration* of chronic atrophic arthritis and the involvement of peripheral nerves. The most severe involvement of the peripheral nerves was seen in cases nos 1, 4 and 5. In case no. 4 no reliable history of the duration of the disease was available; in cases nos. 1 and 5 the duration was 18 and 7 years respectively. In cases nos. 2 and 3 the duration was 16 and 4 years.

Specificity of the Lesions

In order to establish the specificity of the observed morphologic lesions, 86 control cases were examined.

Our control material was studied without knowledge of the clinical findings or of the pathologic diagnosis. The autopsies were done by members of the respective departments of pathology, and the nerves were sent to the neuropathologic laboratory with the autopsy numbers on the containers but without other data. Normal nerves were found in 79 cases. The diseases represented by these cases can be summarized as follows: malignant tumors, 21 cases; vascular diseases (particularly arteriosclerosis, arteriolar sclerosis and hypertension), 23 cases; rheumatic heart disease, 4 cases; neurosyphilis and other organic diseases of the central nervous system (multiple sclerosis, postencephalitic parkinsonism), 5 cases; Buerger's disease, 2 cases; hypertrophic osteoarthritis, 3 cases; miscellaneous diseases such as lobar pneumonia, miliary tuberculosis, subacute bacterial endocarditis, pyelonephritis, chronic nephritis, hydronephrosis, pyosalpinx with consecutive peritonitis, liver cirrhosis (2 cases), peptic or duodenal ulcers (3 cases), volvulus of sigmoid, thyrotoxicosis, polycythemia vera, diabetes mellitus, Gaucher's disease, pemphigus foliaceus and skull fracture, 21 cases. It should be emphasized that in *4 cases* of *chronic rheumatic heart disease* as well as in *3 cases* of *hypertrophic osteoarthritis*, the peripheral nerves were found to be normal.

Lesions were seen in the peripheral nerves of 7 cases. These were 2

cases of chronic atrophic arthritis; 1 of parkinsonian syndrome, probably of postencephalitic origin; 2 of dermatomyositis; 1 of Buerger's disease and 1 of bronchogenic carcinoma. A detailed morphologic study of the findings in the peripheral nerves furnished data for a better differential diagnosis between the nerve changes in chronic atrophic arthritis and in other diseases.

Control Case 4

The patient was a white male, 61 years old, who was first admitted to the hospital on April 14, 1940 complaining of chronic arthritis. The clinical history included a positive Kahn reaction of the blood in 1927; the patient received some antisyphilitic treatment.

Peripheral Nerves. Both femoral nerves were available and 25 blocks were embedded. Only two nodules were found, one in fat tissue between two nerve bundles and another in the perineurium of a nerve bundle. This second nodule was seen grossly in stained preparations. It was more spindle-shaped than the nodules usually are. Serial sections of this nodule showed a three-zonal arrangement; a very small central acellular zone containing homogeneous material, a larger intermediate zone with numerous epithelioid cells and a peripheral zone with numerous, densely packed lymphocytes and plasma cells. The epithelioid cells showed dumbbell-shaped and small elongated nuclei. Somewhat different from perineuritic nodules found in other cases of chronic atrophic arthritis, this nodule was located beneath the outer parts of the perineural sheath of the nerve bundle and directed itself toward the endoneurium (Fig. 7). However, it was clearly seen that the adjacent nerve fibers were separated from the nodule by a thin layer of perineural connective tissue fibers. No invasion of the endoneurium was seen, even in serial sections. An unusual finding was that of one giant cell located in the peripheral part of the nodule; no other giant cells were seen (Fig. 8). There were no degenerative changes in the nerve fibers.

Control Case 46

Illness began in April, 1940 with migratory joint pain in knee, hip and elbow; no swelling or redness. The following findings were noted: generalized muscle aching, some numbness and tingling of lower extremities; generalized vitiligo and chloasma; in the extremities, some interosseous atrophy with a suggestion of clubbing of the fingers; some evidence of atrophy in the lower extremities; foot drop, especially on the right; inability to flex the head; weakness of all muscles in arms, legs, and back. The patient developed respiratory paralysis, which was relieved by respirator ("iron lung"). Tuberculosis with large areas of caseation necrosis was found in an axillary lymph node.

Pathologic Examination

The following pathologic diagnoses were made: dermatomyositis; chronic thyroiditis and extensive destruction of thyroid gland; caseous tuberculosis of axillary and submaxillary lymph nodes and spleen, and miliary tuberculosis of liver and spleen. There was degeneration of muscle fibers with fibrous replacement. Many fibers were swollen and eosinophilic. Lymphocytes and plasma cells were seen in the interstitial muscular tissues and were present in large groups in fat adjacent to the muscle.

Peripheral Nerves. No lesions with either the three-zonal or the two-zonal arrangement, or with the sharp nodular demarcation were seen in the perineurium or epineurium. There were occasional degenerative lesions in the peripheral nerves. The pathologic findings were similar to those described by Kinney and Maher.[2] Single plasma cells were seen scattered between the inflammatory accumulations in the perimysium and toward the perineurium. Areas of the epineurium and the perineurium adjacent to inflammatory foci in the muscles showed accumulations of inflammatory cells.

Control Case 52

The patient presented the following: chronic atrophic arthritis; marked flexion at the hip joints as well as at the knee joints, with considerable atrophic deformity of toes; marked ulnar deviation of hands with marked deformity of all fingers, characteristic of atrophic arthritis; nearly complete destruction of cartilage of both knee joints; partial bony ankylosis with contraction; fixation of patella of both knee joints.

Pathologic Examination

The following pathologic diagnoses were made: hypertensive heart disease; cardiac hypertrophy; marked coronary sclerosis; aortic atherosclerosis; small arteriosclerotic softening in putamen with no other focal lesions in the brain.

Peripheral Nerves. There were changes in the walls of the arterioles in the perineurium and hyalinized thickening and occasional complete occlusion of perineural and epineural arterioles. Singly scattered plasma cells were seen around the blood vessels (perivascularly arranged) but none in the vessel walls themselves. There were no degenerative changes in the nerve fibers.

Control Case 53

The patient presented a history of parkinsonian syndrome starting 3 years previously, with a slight pill-rolling tremor of the hands which gradually increased. There was also trembling of head and jaw, "lead-pipe" rigidity of all extremities and a slight arthritis of the right hand and left shoulder.

Pathologic Examination

The following pathologic diagnoses were made: coronary sclerosis with myocardial fibrosis; cardiac hypertrophy with dilatation; old healed myocardial infarct; bilateral degeneration of the melanin-containing nerve cells in the substantia nigra.

Peripheral Nerves. Both femoral nerves were obtained and 45 blocks were cut, in nearly all of which identical lesions were found. There was a diffusely spread endoneural inflammatory infiltration with lymphocytes (Figs. 9 and 10), an infiltration which followed the course of the endoneural blood vessels. No nodules were found; no zonal pattern was seen. The perineurium was involved only rarely. When it was involved, the same elongated lymphocytic infiltration along the course of the blood vessels was seen. Occasionally degenerative changes in the nerve fibers were found, showing myelin sheath destruction, ballooning of axis cylinders and production of fatty granular material.

Control Case 78

A lower extremity was amputated for thromboangiitis obliterans. The left tibial nerve (from the surgical specimen) was examined. An outstanding feature of this case was the vascular change. Enormous thickening of all layers of the vascular walls (Fig. 11), complete occlusion and occasional subendothelial deposition of homogeneous material were seen. Marked fibrous hyperplasia of the perineurium was found together with massive epineural and perineural accumulations of lymphocytes and numerous plasma cells, usually perivascularly arranged (Fig. 12). Single plasma cells were scattered through the epineurium and the perineurium. No zonal patterns of the lymphocytic accumulations and no nodules were found. The nerve fibers showed various degenerations identical with those reported in 1938 by Barker.[8]

Control Case 81

Both femoral nerves of this case of bronchogenic carcinoma with metastases were examined. They showed infiltration of the epineural fatty tissues with numerous islands of tumor cells but no infiltration of the perineurium or the endoneurium. However, there were definite degenerative changes in the nerve fiber bundles themselves, with myelin sheath degeneration and production of catabolic granules of fat for certain distances along isolated nerve fibers.

Control Case 83

In this patient with dermatomyositis, in muscles as well as in the epineurium and perineurium changes were found identical with those seen in control case no. 46.

The seven control cases with pathologic findings in the peripheral nerves showed various lesions. One case of chronic atrophic arthritis revealed a picture almost identical with that found in our five cases of chronic atrophic arthritis. Another control case of chronic atrophic arthritis showed arteriolar changes in the perineurium of peripheral

nerves with small collections of inflammatory cells located perivascularly but not in the adventitia.

In the case with Parkinson's disease an interesting lesion was found in the peripheral nerves. It showed no nodular pattern and was entirely different from the changes seen in peripheral nerves of cases of chronic atrophic arthritis. The lesion found was designated as diffuse lymphocytic endoneuritis.

In the two cases of dermatomyositis the epineurium and the perineurium were not involved, with the exception of regions in which the adjacent muscles, the perimysium and the interstitial tissues surrounding muscle bundles were diseased. The inflammatory change in epineurium and perineurium was produced by an invasion of the inflammatory process from the neighboring muscular tissues. Again the nodular pattern was absent. This perineuritic change was considered as secondary to the primary myositis.

In the case of thromboangiitis obliterans, vascular changes were seen which were entirely different from the findings in other diseases. The inflammatory lesions were diffusely scattered and particularly marked in the vascular walls and in the perivascular portions. The definite nodular pattern was entirely lacking. The endoneurium was not involved, but definite degenerations of nerve fibers were seen.

In the last case metastatic tumor cells were seen in the epineurium and isolated degenerations of nerve fibers, but no inflammatory cells.

The question arises as to whether the changes in the peripheral nerves in chronic atrophic arthritis are *specific*. There are strong arguments for the conclusion that they are. (1) There is a high incidence of these nerve lesions in cases of chronic atrophic arthritis. They were found in all of 5 cases examined. In 3 of these, they were marked and extensive; in the other 2 cases they were only mild and less characteristic. (2) The lesions are absent in patients suffering from other diseases. In our control material of 86 cases there were 2 cases of atrophic arthritis, 1 with identical findings. Of the remaining 84 cases, 79 showed no pathologic lesions in peripheral nerves. In the 5 cases which showed pathologic changes, the changes were entirely different from those in chronic atrophic arthritis. (3) Distinct location of the nodules in the perineurium or near it in the epineurium, the lack of involvement of the endoneurium, the sharply circumscribed nodular character of the lesions, and the peculiar histologic appearance of the nodules are characteristic and permit differentiating the lesions in chronic atrophic arthritis from nerve lesions in other diseases. (4) These lesions show some similarity to the lesions of the peripheral nerves described in rheumatic fever. Peripheral nerves in rheumatic fever

have rarely been examined. There is only one report. Koeppen[4] (1932) reported changes in the sciatic nerves in three out of seven cases of rheumatic fever. In two cases (one of subacute rheumatic fever with pancarditis and one of rheumatismus nodosus, both with typical Aschoff bodies in the heart muscle) he found circumscribed perivascular lymphocytic infiltrations located in the endoneurium of the sciatic nerve or more rarely in the perineurium with invasion into nerve bundles. In a third case of rheumatic fever the endoneural lymphocytic infiltrations were scarce. In a second group of healed cases of rheumatic fever, among them five cases with healed rheumatic carditis, no lesions in the sciatic nerves were seen. In two cases of arthritis deformans (Table I, case 8; Table II, case 9), one with rheumatismus nodosus and the other with healed endocarditis, the sciatic nerves were found normal. The investigation of Koeppen seems to indicate that there are inflammatory endoneural nodules in the sciatic nerves during the acute or subacute stage of rheumatic fever, nodules which disappear, however, after the acute phase has subsided. There seems to be a discrepancy between the findings of Koeppen and our findings in so far as his examination of the sciatic nerves in two cases of chronic atrophic (postrheumatic) arthritis was entirely negative. (5) There is a striking resemblance between the histologic lesions in the peripheral nerves in chronic atrophic arthritis and the subcutaneous nodules in the same disease. There has been ample discussion about the similarity or dissimilarity between the subcutaneous nodules in rheumatic fever and those in chronic atrophic arthritis (Hawthorne,[5] Findlay,[6] Dawson,[7] Collins,[8] Keil,[9] McEwen,[10] and recently, Bennett, Zeller and Bauer[11]). We are not concerned with this problem, but rather with a comparison of the lesions of the peripheral nerves with the subcutaneous nodules in chronic atrophic arthritis.

The subcutaneous nodules are larger and more irregular in shape than the perineuritic nodules. Notwithstanding this difference, they are alike in that they both show three well-defined zones: the central zone of necrosis, the intermediate zone of proliferating mesenchymal cells and the peripheral ringlike zone of inflammation with lymphocytes and plasma cells. In perineuritic nodules the central area of necrosis is not very well developed; there is more often a small "fibrinoid" area containing nuclear débris rather than a large necrotic homogeneous mass. Conglomeration into larger areas as seen in subcutaneous nodules has not been found in perineuritic nodules. There is an occasional loosening of the intercellular substance in the central zones of perineuritic nodules but not the lattice formation seen in subcutaneous nodules. Palisading of epithelioid cells in the intermedi-

ate zone is not found in the perineuritic nodules. There is a symplasm of cells, and multinucleated cells and mitotic figures are rare or absent. The peripheral zone in both subcutaneous and perineuritic nodules is characterized by diffuse lymphocytic infiltration.

The conclusion seems to be justified that the reported lesions of circumscribed perineuritis are pathologically specific for chronic atrophic (rheumatoid) arthritis. The pathologic state must be classified as circumscribed perineuritis, or better as *rheumarthritic nodulous perineuritis*. The attribute "nodulous" has been chosen because a multiplicity of nodules is seen with completely circumscribed demarcation of the single nodule. *Nodulosus* (Latin) means rich in nodules, while "nodular" means to have the characteristic appearance of nodules. "Rheumarthritic" is an abbreviation of rheumatoid arthritis.

Clinical Discussion

Pemberton,[12] in his monograph, considered painful symptoms in arthritis due to an involvement of nervous tissues to be either primary or secondary to pathologic processes in adjacent regions. In chronic atrophic arthritis there are pains and paresthesias, vasomotor, secretory and trophic changes in the skin. There is the characteristic clinical picture of diseased bones and joints. As the disease progresses there is muscular atrophy. Pains and paresthesias, such as numbness, often follow the course of a peripheral nerve. Paresthesias other than numbness are the feeling of gloves or of cotton or muslin, pricking, tingling, stabbing, gnawing, or tearing. The pains and paresthesias are increased by touch or pressure on the nerve and by contraction of the muscles. These are symptoms of neurologic disorder. However, neurologic examination of our cases as well as of those reported in the literature showed no disturbances of sensations—touch, pinprick, heat and cold and the deep sensory functions.

There is a striking parallel between the occurrence of pain and paresthesias in chronic atrophic arthritis and in so-called polyneuritis, particularly in regard to their distribution. Polyneuritis usually shows a symmetric bilateral distribution and a more severe clinical involvement of the distal parts of the extremities. Chronic atrophic arthritis commonly shows bilateral symmetry in the form of a marked bilateral involvement especially of distal parts; the hand and fingers, as well as the feet and toes. The involvement of the shoulders in chronic atrophic arthritis amounts to only 9 per cent (Kuhns [13]). When found, it is usually bilateral.

Cutaneous vasomotor, secretory and trophic disorders are not rare in polyneuritis. During the first World War, bone and joint changes,

due to injuries to the peripheral nerves only, were frequently observed,[14] and loss of reflexes and paralysis are common in clinical polyneuritis. In chronic atrophic arthritis, however, the reflexes may be exaggerated. It may be incorrect to emphasize an analogy between chronic atrophic arthritis and polyneuritis, for the muscular degeneration seen in interosseous atrophy in chronic atrophic arthritis (for example in the small muscles of the hands and fingers) is different from the degeneration of muscles seen in polyneuritis. The reaction of degeneration of muscles and fibrillary twitchings are usually not found in chronic atrophic arthritis. Hence muscular lesions in this disease cannot be explained by pathologic changes in the peripheral nerves. Pemberton [12] explained the muscular atrophy in chronic atrophic arthritis by a proliferation of the interstitial connective tissue in and around the muscle bundles and a resultant compression with degeneration of the muscular tissue. Later in the disease, said Pemberton, the muscular tissue becomes largely "displaced and replaced by the newly formed fibrous tissue." Thus, muscular atrophy in chronic atrophic arthritis may be due to injury not originating in the peripheral nervous tissues. The articular and periarticular pathology, the synovial and bony changes and the swelling of the periarticular soft parts seem to be of primary character. So, muscular, articular and periarticular lesions in chronic atrophic arthritis may not be due to peripheral nerve lesions. There remain, however, the pains and paresthesias in chronic atrophic arthritis and the vasomotor, secretory and trophic cutaneous symptoms in this disease which show a definite parallel with those in so-called polyneuritis.

Pemberton [12] has already suggested that the *nervous tissues* in chronic atrophic arthritis *must* be affected in order to give rise to painful symptoms. We now find that there are definite morphologic changes in the peripheral nerves of patients with chronic atrophic arthritis in that the perineurium of the trunks of the peripheral nerves shows a rather regular involvement. We are inclined to consider this change responsible for the pains, paresthesias and trophic clinical symptoms. The examination of the peripheral nerve bundles for degenerative changes of myelin sheaths and axis cylinders was completely negative. Consequently, the sensory and trophic disturbances can be related only to the perineuritic pathologic changes.

There is another strong argument for the correlation of the perineuritic nodules with the pains, paresthesias and trophic disorders. Perineuritic lesions usually cause irritation of the nerve fibers first, whereas endoneural lesions may produce degeneration of nerve fibers. Symptoms of irritation are pain, paresthesias, and vasomotor, secretory and trophic disorders, but symptoms of endoneural lesions with

loss of function are objective sensory changes and degenerative muscular atrophy. Another point of interest is the migratory and transitory character of the pains and paresthesias which favors again a perineural location more than an endoneural. Thus it seems justifiable to correlate some of the clinical findings in chronic atrophic arthritis with anatomic lesions in the peripheral nerves, *i.e.*, with rheumarthritic nodulous perineuritis. On the other hand, the articular pathologic changes are of primary character, and the involvement of the joints is independent of the perineuritic lesions. Synovial and perineuritic changes should be considered as concomitant changes, possibly due to the same agent.

Pathogenesis

As already mentioned, the pains and paresthesias are considered a consequence of the multiple inflammatory nodules in the perineurium of the peripheral nerves. There is reason enough to assume an irritation of nerve fiber bundles by the space-consuming extension of the nodules, or by other factors not yet known. At any rate, the pathogenesis of pains, paresthesias, and vasomotor, secretory and trophic disorders is now better understood after finding inflammatory perineural nodules in the peripheral nerves.

The pathogenesis of rheumarthritic nodulous perineuritis itself remains obscure. Certainly the perineuritic nodules are not in contact with regions of inflammation in the synovia and other parts of the joints. The changes in the joints and in the peripheral nerves are independent. They may be produced by a common etiologic source, but both are due to the effect of the noxious agent *in situ*.

The perineuritic lesions are also independent of subcutaneous nodules. In our five cases with perineuritic lesions, there were no subcutaneous nodules and the perineuritic nodules were sharply circumscribed and showed no tendency to grow into neighboring tissue structures and to coalesce with each other. These inflammatory foci are of autochthonous origin; they are produced by an infectious or a toxic agent or by a combination of infectious and toxic agents *in situ*.

The perineural inflammatory nodules represent a peculiar type of *active* tissue reaction. It is of a type similar to but not identical with that seen in tuberculosis, syphilis, leprosy and other chronic infectious diseases. The question arises whether the perineural inflammation seen in our cases of chronic atrophic arthritis was produced by the agent of chronic atrophic arthritis or by another and unrelated infectious or toxic agent. The five cases of chronic atrophic arthritis in which the peripheral nerves could be examined showed no conspicuous *chronic*

infectious disease either clinically or at autopsy. One patient (case no. 1) died from fat embolism as a consequence of orthopedic manipulation; the autopsy revealed chronic rheumatic heart disease with mitral valvulitis and pericardial adhesions but no other chronic infectious disease. In case no. 2 lobar pneumonia was the cause of death, and there was coronary sclerosis with hypertension but no chronic infectious disease. Another patient (case no. 3) died from acute septicemia due to hemolytic streptococci. In case no. 4 healed pulmonary tuberculosis and chronic salpingitis were found. In case no. 5 death was due to acute septicemia caused by hemolytic streptococci. Acute bacterial mitral valvulitis and myocarditis, chronic bilateral pyelonephritis and generalized amyloidosis were found. There remain at least the two cases, nos. 1 and 2, in which the findings of perineuritic nodules can be explained only on the basis of chronic atrophic arthritis because no other acute or chronic inflammatory disease was present.

At least three of the eleven cases revealed rheumatic inflammatory processes in the heart (mitral valvulitis) at autopsy, that is, about 27 per cent. A high incidence of rheumatic damage to the heart is to be expected in cases of chronic atrophic arthritis if there is a causative relationship between these diseases. The concomitant finding of rheumatic valvulitis, chronic atrophic arthritis and rheumarthritic nodulous perineuritis in the same body without the presence of any other inflammatory disease, as in case no. 1, points strongly to a common source of the inflammation involving the three different structures; namely, the heart, synovia and peripheral nerves. It is highly improbable that the perineuritic nodules are due to any other agent of inflammatory disease than that of chronic atrophic arthritis.

There are three prominent features of rheumatic pathologic morphology which have to be mentioned: the rheumatic nodules in the heart (Aschoff bodies), the subcutaneous nodules and the "fibrinoid change." Aschoff bodies were not seen in the perineuritic lesions. Bennett, Zeller and Bauer [11] emphasized the finding of a few round or oval lesions of the size of miliary tubercles bearing "a distinct resemblance" to myocardial Aschoff nodules in the subcutaneous nodules of cases of *rheumatic fever*. However, in subcutaneous nodules of cases of *chronic atrophic arthritis*, Aschoff-like nodules were "rarely if ever" seen. None were observed by these investigators in the 67 subcutaneous nodules of atrophic arthritis examined by them. Aschoff,[15] in 1939, considered the rheumatic heart nodule as specific for the granulomata which occur in rheumatism in general. Rheumatic nodules, according to Aschoff, are cell formations specific to rheumatic fever in which they are exclusively seen. Baggenstoss and Rosenberg,[1] investigating cardiac lesions in

chronic rheumatoid arthritis, found in 3 cases with subacute cardiac lesions and in 5 out of 7 cases with chronic cardiac lesions, typical myocardial Aschoff bodies. Thus it is definitely established that Aschoff bodies are found in a rather high incidence in the hearts of cases of chronic atrophic arthritis. They are not seen in the subcutaneous nodules or in the perineuritic nodules. Subcutaneous nodules in chronic atrophic and perineuritic nodules in the peripheral nerves in the same disease are in their morphologic appearance different from Aschoff bodies, yet they are disease-specific.

The "fibrinoid" degeneration of Klinge [16] and the "mucinous edema" of Talalajew [17] are two terms for the same pathologic phenomenon. It consists of swelling of collagen bundles of fibrous tissue and an exudation of homogeneous material into intercellular spaces. This is a correct observation beyond any doubt. However, the conclusions in regard to the histochemical character, specificity and significance of this change vary greatly. Klinge considered the fibrinoid change as the early primary change in all rheumatic diseases, followed by focal degeneration, necrosis, mesenchymal proliferation and invasion by inflammatory cells. Collins [8] considered the "fibrinoid" change a prominent and characteristic feature of the rheumatic lesion. However, he called this change the basic type of tissue reaction in states of hypersensitivity. According to Bennett's [18] opinion, the term "fibrinoid degeneration" has received more attention than it probably deserves. He believed that this type of reaction frequently represents a nonspecific type of inflammatory change in connective tissue in which the collagen fibers are separated by exudate. He preferred the term "plasma soaking" to that of fibrinoid degeneration. For rheumatic nodules in the heart, Aschoff [15] himself declared it in no way essential that the formation of the nodules be preceded by fibrinoid degeneration of the intercellular substance.

In our own experience, "fibrinoid degeneration" is not histologically specific for a rheumatic lesion. An identical type of reaction, for example, can be seen in Buerger's thromboangiitis obliterans, particularly in the subendothelial layers of vascular walls. We consider the intercellular and interfibrillar exudation of first liquid and finally genuine coagulating fibrin or fibrinlike (fibrinoid) material as nonspecific for the rheumatic lesion notwithstanding the fact that it is of common occurrence in this disease. No matter which view is accepted in regard to the specificity of the fibrinoid change, there remains the fact that in the subcutaneous nodules of rheumatic fever and of chronic atrophic arthritis, in the Aschoff bodies and in rheumarthritic nodulous perineuritis, granulomata of a peculiar and specific character are found. The histologic appearance of these granulomatous nodules varies in respect

to the stage of the disease and varies in so far as the specific tissue reaction (subcutis, heart, perineurium) interferes with the potentialities of general defense against the agent.

In principle, the perineuritic nodule in chronic atrophic arthritis shows features identical with those in other rheumatic tissue lesions. There is the "plasma soaking" (Bennett) type of exudation (fibrinoid degeneration or fibrinoid change), the proliferation of mesenchymal (epithelioid) cells and the peripheral infiltration with lymphocytes and plasma cells. There is a division of these histologic characteristics into two or three merging zones.

The pathogenesis of chronic atrophic arthritis is not known. It may be an etiologically uniform disease, or there may be various disease entities producing identical clinical pictures. However, there is a strong possibility that the majority of cases diagnosed as chronic atrophic arthritis are of the same etiology. In the literature two different opinions are advanced. One opinion is that chronic atrophic arthritis is a consequence of previous rheumatic fever, either as a late stage of this disease or as a causally related disease. The other opinion is that chronic atrophic arthritis has nothing to do with rheumatic fever. A definite decision between these two opinions cannot be reached before the agents of both diseases are known.

Recent studies suggest that there is a close relationship between chronic atrophic arthritis and previous rheumatic fever. We refer again to the study of Baggenstoss and Rosenberg [1] concerning cardiac lesions in cases of chronic atrophic arthritis and to the investigation of Bennett, Zeller and Bauer.[11] Baggenstoss and Rosenberg, in examining 25 cases of chronic atrophic arthritis, found cardiac lesions in 20, among them 14 with lesions resembling those produced by rheumatic fever. The rheumatic lesions were *grossly* demonstrable in all but 1 of the 14 cases. Baggenstoss stated that there is a definite relationship between rheumatoid arthritis and rheumatic fever. Bennett, Zeller and Bauer consider the subcutaneous nodules in rheumatic fever and in rheumatoid arthritis as disease-specific and the nodules in both diseases as presenting similar or identical structural and cytologic features.

Rheumarthritic nodulous perineuritis adds one more link to this chain of relationship between rheumatic disease and chronic atrophic arthritis. Since the lesions in the peripheral nerves represent a definite inflammatory change of specific character and since other sources of inflammation or infection can probably be excluded, there remains only one explanation: the perineuritis in chronic atrophic arthritis belongs to the same group of pathologic lesions as are illustrated by the synovial and periarticular inflammation and thickening, the inflammatory and

proliferative nodular changes in the subcutis and the frequent cardiac stigmata of rheumatic type in this disease. This group of lesions indicates a close relationship of chronic atrophic arthritis to rheumatic fever, or better, rheumatic disease, including rheumatic polyarthritis, rheumatic endocarditis, valvulitis, myocarditis and other rheumatic manifestations.

Summary

1. In 3 out of 5 cases of chronic atrophic (rheumatoid) arthritis, characteristic pathologic lesions were found in peripheral nerves. The remaining 2 cases showed only mild lymphocytic inflammation.

2. The location of the pathologic lesions in the perineurium and their sharply nodular and peculiar granulomatous morphology are features of a disease-specific appearance.

3. By examination of peripheral nerves from 86 control cases the specificity of the findings in peripheral nerves of patients with chronic atrophic arthritis has been confirmed. Seven control cases showed pathologic lesions. Two of these were cases of chronic atrophic arthritis, 1 of which showed lesions similar to those found in the 3 cases of chronic atrophic arthritis and the other showed only arteriolar changes in the peripheral nerves. The remaining 5 control cases were 1 of Buerger's disease, 2 of dermatomyositis, 1 of postencephalitic parkinsonism and 1 of carcinomatous infiltration of the epineurium. The lesions of the peripheral nerves in the nonarthritic control cases differed distinctly from the perineuritic changes in chronic atrophic arthritis, even when there was an accompanying inflammatory or infectious disease.

4. The lesion in the peripheral nerves of cases of chronic atrophic arthritis is best designated as "nodulous rheumarthritic perineuritis."

5. Nodulous rheumarthritic perineuritis should be considered a specific active inflammatory process concomitant and parallel with other rheumatic pathologic features in chronic atrophic arthritis, such as rheumatic heart disease, subcutaneous nodules and synovial inflammation and proliferation.

Note: We are indebted to C. Graham Eddy for his photomicrographic work.

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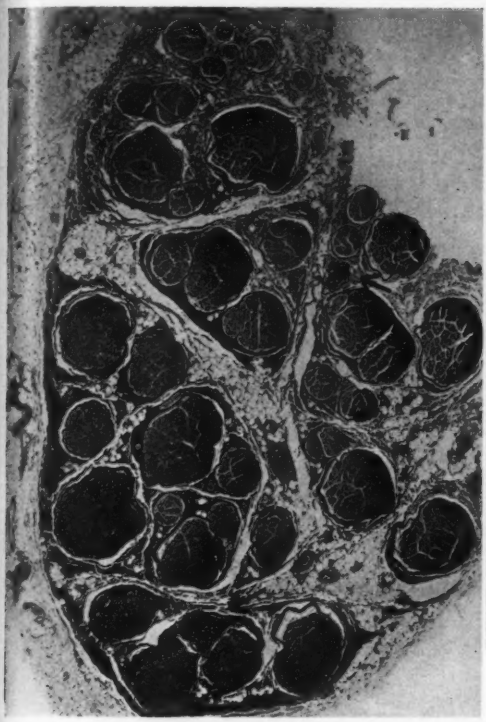
DESCRIPTION OF PLATES

PLATE 129

FIG. 1. Case 1. Cross section through the tibial nerve. Two distinct nodules are marked by arrows. Hematoxylin and eosin stain. × 19.

FIG. 2. Case 1. Femoral nerve. The different zones of one nodule are clearly seen: a central acellular zone with homogeneous material, nuclear débris and a few dark granules; a small intermediate zone consisting of cells with large irregularly shaped hypochromatic nuclei, and an outer peripheral zone of lymphocytes and plasma cells. The nodule is located in the perineurium between two nerve bundles. Hematoxylin and eosin stain. × 150.

FIG. 3. Case 1. Higher magnification of the nodule shown in Figure 2. Hematoxylin and eosin stain. × 420.



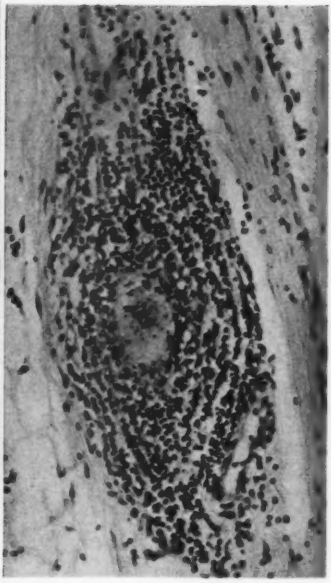


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Plate 130

Fig. 4. Case 1. Perineuritic nodule as seen on the lower portion of Figure 1. A thickened arteriolar wall and a small amount of acellular substance in the adventitia are seen. Hematoxylin and eosin stain. × 175.

Fig. 5. Case 4. Femoral nerve. A perineuritic nodule is shown in which the central acellular zone is absent. There are a number of epithelioid cells in the intermediate zone with numerous collagenous fiber bundles giving a somewhat looser structure. Hematoxylin and eosin stain. × 110.

Fig. 6. Case 1. Intermediate zone of a perineuritic nodule. The epithelioid cells show a faintly stained cytoplasm and the demarcation of single epithelioid cells from each other is ill-defined. In the lower right portion of the field, cells of the lymphocytic ring and a few plasma cells are seen. Cresyl-violet stain. × 575.

Fig. 7. Control case 4. Chronic atrophic arthritis. Femoral nerve. Spindle-shaped perineuritic nodule with unusual development toward the endoneurium, but without invasion into the endoneurium. At the top of the picture, one giant cell is seen. Hematoxylin and eosin stain. × 150.

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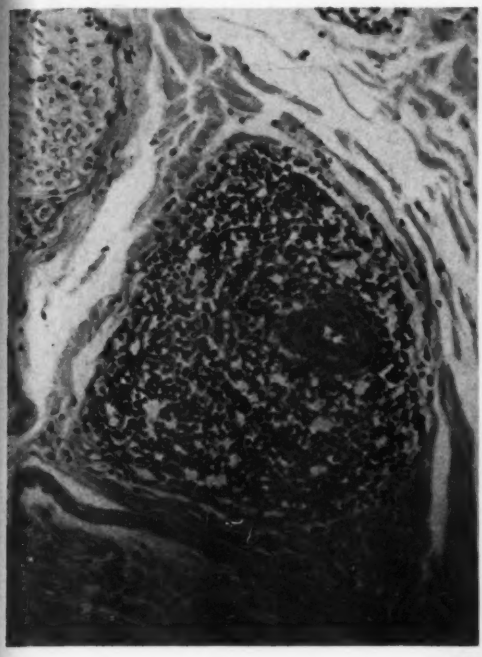

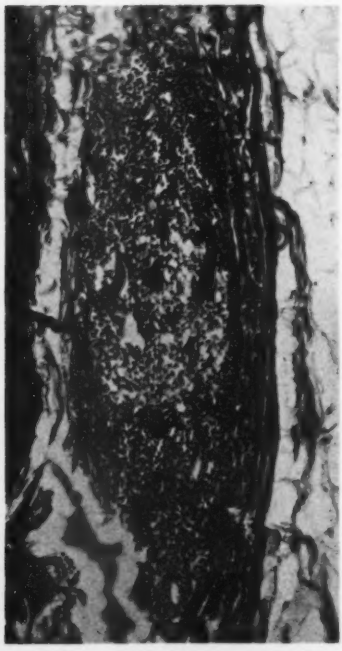

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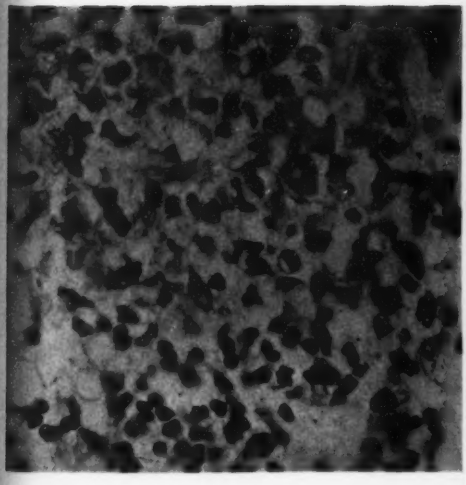

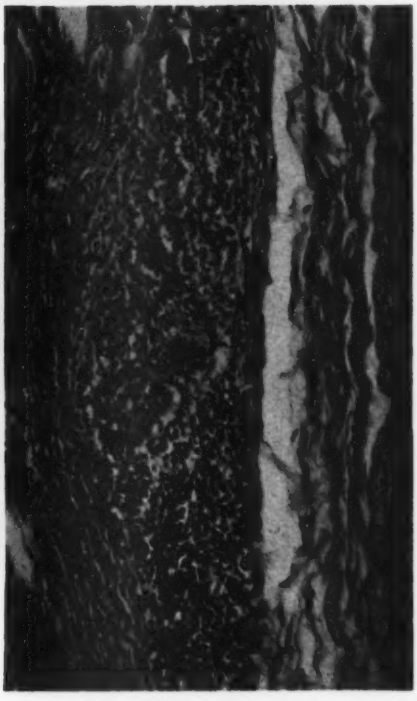

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PLATE 131

FIG. 8. Higher magnification of the upper portion of Figure 7. × 875.

FIG. 9. Control case 53. Parkinsonian syndrome. Femoral nerve. Diffuse endoneural and moderate perineural lymphocytic infiltration. Hematoxylin and eosin stain. × 50.

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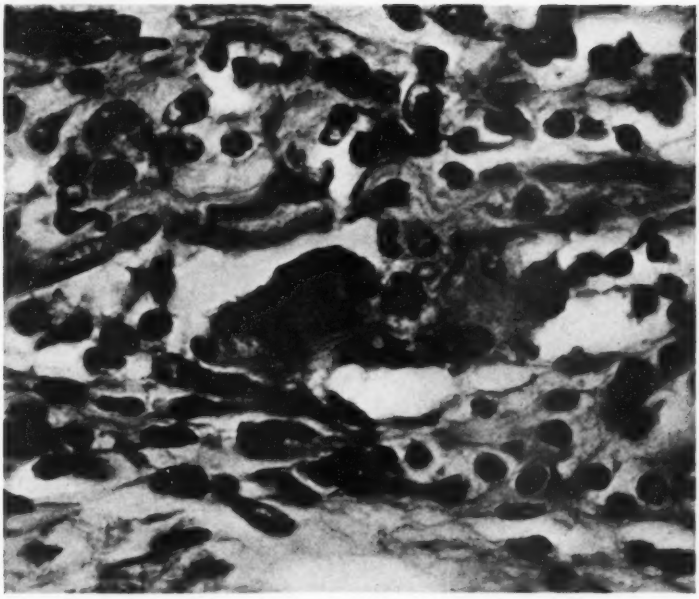

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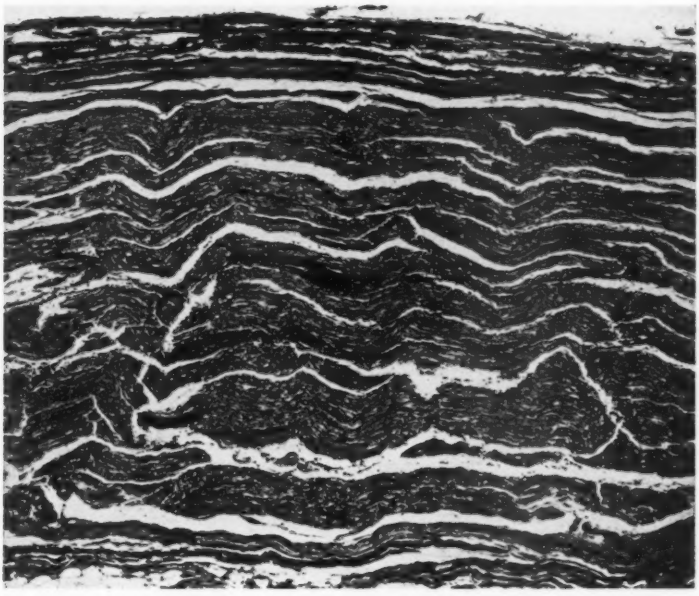

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PLATE 132

FIG. 10. Higher magnification of one of the areas of lymphocytic infiltration in the femoral nerve used for Figure 9. Hematoxylin and eosin stain. × 175.

FIG. 11. Control case 78. Left tibial nerve. Thromboangiitis obliterans. Marked thickening of the wall of a small artery is seen with perivascular fibrosis. Hematoxylin and eosin stain. × 110.

FIG. 12. Control case 78. Left tibial nerve. Thromboangiitis obliterans. Massive perineural accumulation of lymphocytes and plasma cells without a nodular pattern. Hematoxylin and eosin stain. × 110.

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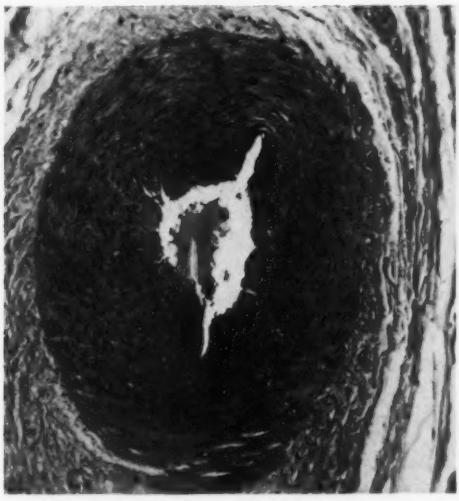

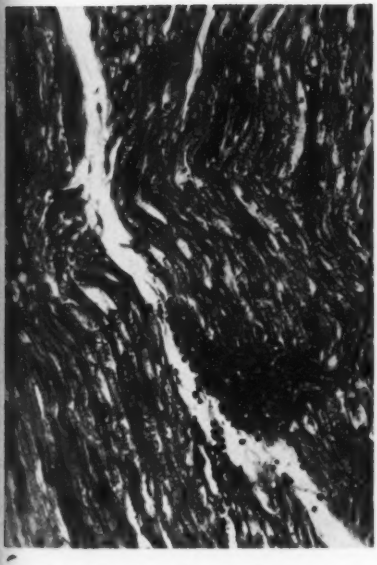

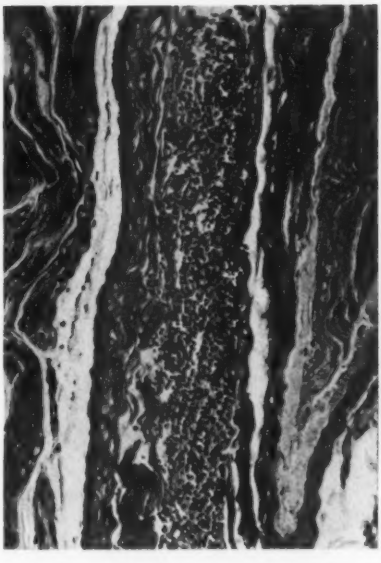

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Peripheral Nerves in Chronic Arthritis

EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY*

V. Effects of Intravenous Injections of Solutions of Gum Arabic, Egg Albumin and Gelatin Upon the Blood and Organs of Dogs and Rabbits

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During the course of experiments with colloidal solutions of several macromolecular substances (polyvinyl alcohol, methyl cellulose) which were tested for their suitability as nonhematogenous blood substitutes, it was observed that they give rise to the development of atheromatous lesions and foam-cellular storage phenomena in various organs, resembling closely those produced by hypercholesterolosis. It was concluded from this evidence and from observations made with vasculotonic agents that degenerative arterial lesions may be elicited by any one of three distinct types of causative mechanisms: (1) vasculotonic agents, causing either a hypertonic ischemic hypoxemia of the vascular wall or a hypotonic stagnant hypoxemia of these tissues; (2) excessive hydrostatic pressure in the vascular lumen resulting from an excessive filling of the vessels with blood (plethora) and producing a hypoxemia of the vascular tissues by an overextension of the vascular wall with collapse of the vasa vasorum and by a compression of the elastic and contractile media against the rigid adventitia; (3) disturbances of the colloidal status of the blood plasma elicited by abnormalities in the quantitative and qualitative physicochemical conditions in this medium and impairing an adequate exchange of gaseous and nutritive elements between the plasma and the vascular tissues by the formation of films or precipitates in the interface (Hueper; Hueper and Landsberg; Hueper, Landsberg and Eskridge; Martin and Hueper; Hueper and Ichniowski).

In further elaboration of these investigations, dogs and rabbits were injected intravenously at frequent intervals with hypertonic solutions of the following three macromolecular colloids: gum arabic, gelatin and ovalbumin. These substances were chosen for the following reasons. Solutions of gum arabic are used extensively in the treatment of nephrotic hypoproteinemia and are known to produce colloidal disturbances of the blood (acute leukopenia, reduction in number of erythrocytes and in amount of hemoglobin, hastened erythrocytic conglomeration and sedimentation, lengthened bleeding and coagulation time, decrease of plasma proteins) and storage phenomena in internal organs including

* Received for publication, January 5, 1942.

the liver, spleen, lymph nodes, kidney and bone marrow (Yuile and Knutti; Heckel, Erickson, Yuile and Knutti; Studdiford; Frimberger; Dick, Warweg and Andersch; Jackson and Frayser; Fukuhara and Hiratsuka; Onozaki and Sanada; Andersch and Gibson; Metcalf and Hawkins; Hall, Gibson and Weed; Falkenstein and Jackson; Christie, Phatak and Olney; Keith, Power and Wakefield; Erickson, Heckel and Knutti), which resemble closely those elicited by polyvinyl alcohol and methyl cellulose.

Clinical as well as experimental observations (Heinlein; Heinlein and Muschallik; Schmitt; Holman; Dick and Leiter; Werley) suggest that abnormalities in the colloidal equilibrium and reactivity of the blood proteins are involved in the production of degenerative and proliferative arterial lesions, and of the colloidoclastic hematic reactions associated with allergic or related responses (hyperglobulinemia, hastened erythrocytic sedimentation and amyloidosis). A study of the relationship between chronic disturbances of the colloidal status and composition of the plasma proteins, induced experimentally by the intravenous injection of foreign proteins (gelatin and ovalbumin), and the development of arteriosclerosis seemed to be indicated, especially as gelatin solutions and modified bovine plasma albumin have been recommended recently for the treatment of shock (Hogan; Taylor and Waters; Cohn; Keys, Taylor and Savage).

Experimental Investigation

I. Gum Arabic

The gum arabic solution used in the experiment was prepared as follows: High-grade gum arabic tears, ground into a fine powder, were dissolved in physiological saline solution by heating on a water bath, giving a moderately opaque, viscous liquid. This material, which contains impurities, was mixed with a large quantity of "Hyflo Supercel"* and filtered while hot through coarse filter paper in a Büchner funnel. The filtrate was almost clear and slightly brown. A 25 per cent solution of gum arabic thus prepared had a pH of about 4. Fifteen to 20 cc. of lime water with a pH of 10 were needed to neutralize 80 to 100 cc. of this solution, reducing thereby its concentration to about 20 to 22 per cent. This neutralized material remained fairly clear after autoclaving for 10 to 12 minutes under 14 to 15 lbs. of pressure, while a moderately heavy precipitate was formed when the time of sterilization was extended to 30 to 40 minutes. This was accompanied by a

* Hyflo Supercel is a preparation of purified diatomaceous earth, obtained from the Dicalite Co., 120 Wall Street, New York, N. Y.

distinct reduction in the viscosity of the solution, which also decreased somewhat after moderate autoclaving (Gartside). Viscosity determinations made on gum arabic solutions after autoclaving for 10 to 12 minutes gave the following results: a 3.5 per cent solution had a viscosity of 2.04 at a temperature of 35° C.; a 5 per cent solution, 2.53; a 10 per cent solution, 5.06, and a 20 per cent solution, 18.4 (readings by Hess's viscosimeter).

Eight dogs and two rabbits were used in the experiment. The dogs weighed between 7.12 and 9.17 Kg., and the rabbits weighed approximately 2.5 Kg. at the start of the experiment.

The injection of from 20 to 50 cc. of the 25 per cent solution of gum arabic into the ear veins of rabbits was tolerated without any immediate, untoward symptomatic effects. When, on the other hand, 30 cc. or more of this material were injected into the jugular veins of dogs, these animals invariably reacted with excessive salivation, vomiting and defecation, which appeared 2 to 6 minutes after the introduction of the gum arabic solution. The severity of this reaction was reduced and consisted usually only of excessive salivation, when the amount and the concentration of the gum arabic solution injected was decreased to 10 to 20 cc. of a 10 per cent solution. It was noted that during the course of repeated intravenous injections of gum arabic solution several dogs developed a severe transitory edema of the head, especially in the region of the jaws. The dogs which died exhibited a rapid and marked loss of weight, while those surviving to the end of the experiment maintained their weight.

To study the reactions elicited by the intravenous introduction of gum arabic solution into dogs, 10 cc., 20 cc. and 30 cc. of a 25 per cent solution were injected into the jugular veins of three animals, respectively, and blood studies were made at various intervals thereafter (5 minutes, 15 minutes, 45 minutes, 2 hours, 4 hours, 6 hours, 24 hours, and 2, 3, 7, 8, 10 and 14 days). These three dogs, together with five additional dogs, were then placed upon a regime consisting of daily injections of a gum arabic solution. The pertinent data are summarized in Table I.

The individual doses of gum arabic solution administered to these dogs varied between 20 cc. of a 10 per cent solution to 80 cc. of a 25 per cent solution. The higher doses were given usually toward the end of the experiment and to dogs surviving for more than 75 days.

The two rabbits were injected with a gum arabic solution, the concentration of which was gradually increased from 6 to 25 per cent. One rabbit received 10 such injections within 2 weeks, while the second rabbit received 40 injections within 8 weeks. Both animals died.

Reactions of the Blood after a Single Injection of Gum Arabic Solution

The cellular and physicochemical reactions of the blood of the three dogs after a single injection of 10 to 30 cc. of a 25 per cent solution of gum arabic were identical in character, but varied in intensity with the amount of gum arabic solution administered. They may be summarized as follows:

There developed immediately after the injection a marked but transitory leukopenia and especially granulocytopenia, lasting from 1 to 2

TABLE I
Gum Arabic Solutions Injected into Dogs

Dog no.	Gum arabic solutions injected (total amount)	Gum arabic dry weight	Number of injections	Time of treatment	Death and time after last injection
		(gm.)		(days)	
729	10% sol., 500 cc. 25% sol., 1,360 cc.	390	47	90	Killed 2 days after last injection
708	10% sol., 840 cc. 25% sol., 750 cc.	274	52	35	Died 5 days after last injection
712	10% sol., 470 cc. 25% sol., 1,460 cc.	415	46	90	Killed 2 days after last injection
701	10% sol., 880 cc. 25% sol., 860 cc.	303	54	78	Killed 25 days after last injection
696	10% sol., 120 cc.	12	4	5	Died on 6th day of experiment
698	10% sol., 240 cc. 25% sol., 90 cc.	43	11	21	Died on 21st day of experiment
605	10% sol., 570 cc. 25% sol., 2,160 cc.	597	31	110	Killed 5 weeks after last injection
681	10% sol., 240 cc. 25% sol., 1,530 cc.	406	47	90	Killed 7 weeks after last injection

hours. This response was followed by a moderate granulocytosis of 2 hours' duration in the presence of a normal number of leukocytes. Numerous immature, granular leukocytes were present in the peripheral blood during this phase. A completely normal leukocytic blood picture was found 72 hours after the injection and was maintained thereafter.

The number of erythrocytes dropped sharply during the leukocytic phase and returned only gradually within the following 72 hours to the original level. Numerous nucleated erythrocytes (20 to 25 per 100 leukocytes) were present during the erythropenic phase. The volume of packed blood cells and the amount of hemoglobin underwent corresponding changes. The sedimentation rate of the erythrocytes was markedly accelerated during the first 24 hours and gradually returned to a normal level within 6 days. The coagulation time was moderately lengthened during the first hour and became moderately shortened thereafter for several days. The prothrombin time remained within normal limits. The viscosity of the plasma was unchanged, while the viscosity of the whole blood became moderately reduced during the period of erythropenia.

Table II illustrates the changes observed in the dog which received 20 cc. of the 25 per cent gum arabic solution.

Reactions of the Blood after Repeated Injections of Gum Arabic Solution

Following the repeated intravenous introduction of gum arabic solution, changes developed in the blood which followed a certain pattern and sequence and which were subject to some individual variations.

TABLE II

Observations on the Blood after a Single Injection of Gum Arabic Solution

Elapsed time	Hemoglobin	Erythrocytes	Leukocytes	Polymorphonuclear leukocytes	Sedimentation rate (mm.)
	18.0	7,730,000	15,400	69	
5 minutes	17.7	7,800,000	7,600	16	24.5
15 minutes	17.9	7,630,000	4,800	14	10.0
45 minutes	16.0	7,260,000	4,300	58	31.0
2 hours	16.0	7,300,000	10,700	84	7.0
4 hours	15.1	6,480,000	8,200	88	6.0
6 hours	14.9	6,870,000	7,200	88	32.0
8 hours	16.4	6,930,000	8,500	83	13.0
24 hours	15.3	6,960,000	9,800	76	30.0
6 days	16.3	7,990,000	14,800	66	1.0
8 days	17.0	8,220,000	14,700	82	2.0
10 days	15.5	6,770,000	10,400	60	1.0

There occurred a gradually increasing and finally severe reduction in the number of erythrocytes, in the volume of packed red blood cells and in the amount of hemoglobin. The sedimentation rate of the erythrocytes became progressively accelerated and ultimately reached a high value. The prothrombin time did not vary appreciably, while the coagulation time was usually moderately to markedly shortened and only occasionally moderately lengthened. The viscosity of the whole blood showed a tendency to decrease because of the increasing anemia, whereas the viscosity of the plasma became simultaneously elevated, reaching pathological value only rarely, however, during the final stage of the treatment, when massive amounts of a highly viscous gum arabic solution were given.

The number of leukocytes underwent a considerable reduction at times and at other times reached values of 300 per cmm. The counting of these cells was severely interfered with during the periods of intensive treatment by the formation of a granular precipitate in the blood treated with acetic acid. Following an initial agranulocytic leukopenic stage, the leukopenia was characterized by a relative increase of the granular leukocytes which sometimes represented up to 97 per cent of the total number of cells identified. The polymorphonuclear leukocytes

were especially numerous when the total leukocytic count was low. There was, moreover, a moderate to marked increase of the immature forms. It is remarkable that during periods when the treatment with gum arabic solution was temporarily interrupted, or during the recovery period following the cessation of treatment, there occurred a moderate leukopenia characterized by a marked decrease of the polymorphonuclear leukocytes. It is furthermore noteworthy that the reported blood changes underwent a relatively rapidly progressing recovery within a few days after the cessation of even a very severe and prolonged treatment. Table III may serve as a representative illustration of the changes in the blood observed after repeated injections.

TABLE III

Changes in the Blood after Repeated Injections of Gum Arabic Solutions

Time	Gum acacia; total amount	Hemoglobin	Erythrocytes	Leukocytes	Polymorphonuclear leukocytes	Sedimentation rate
	(gm.)					(mm.)
1st day	7.5	15.2	6,350,000	15,400	79	
3rd day	15.0	15.9	6,300,000	13,100	69	1.0
7th day	30.0	16.0	6,520,000	6,800	57	23.0
14th day	68.75	11.8	5,650,000	4,200	80	48.0
18th day	117.50	9.4	3,300,000	1,200	78	45.0
25th day	133.75	8.5	3,650,000	3,300	96	65.0
35th day	133.75	11.9	5,470,000	9,500	82	10.0
55th day	186.75	10.8	4,790,000	6,100	40	29.0
76th day	261.75	8.3	4,140,000	3,600	87	65.0
84th day	301.75	4.5	2,020,000	impossible to count	95	81.0
109th day*		10.7	5,230,000	6,400	53	8.0

* Injections discontinued after 87th day.

Morphological Pathology

The two dogs which died 5 days and 21 days after the start of the experiment showed only a hemorrhagic pneumonia. The other organs were normal in size and appearance. The rabbit which died after 2 weeks of treatment exhibited the same pulmonary pathology in addition to a firm, yellow-brown spleen surpassing by about ten times the size of a normal organ.

The dog which died 40 days after the start of the experiment revealed compression atelectasis of both lungs due to a turbid, reddish fluid containing fibrinous shreds in the pleural cavities. A shallow ulceration, measuring 2 cm. in diameter, was found in the duodenal mucosa about 2 cm. below the pylorus. The liver was huge, weighing 1010 gm., and had a red-yellow mottled surface and a firm consistency. The spleen weighed 48 gm. and was of red color and firm consistency. The other internal organs, including the brain, were normal.

The postmortem examination of the animals which survived longer

(90 to 115 days) and which were killed at the end of the experiment, or up to 25 days later, invariably showed an excessively enlarged brown-red liver with yellow spotting, varying in weight from 1004 to 1150 gm. The spleen was moderately enlarged, weighing from 55 to 85 gm. The abdominal lymph nodes were also moderately enlarged, while the kidneys were swollen and had a grayish brown color.

Histological examination of the organs showed the following changes attributable to the treatment given: In the three animals (two dogs and one rabbit) which died within the first 21 days of the experiment, the main lesions were located in the liver, which showed a moderate foam-cellular transformation of the parenchymatous cells. It was noted that the marginal areas of the liver cells were homogeneous and very much denser than the central portions, thereby causing a sharp delineation of the liver cell cords from the surrounding distended and edematous sinusoids. The renal glomeruli often exhibited a broadened and homogeneous matrix, while the tubular epithelium was vacuolated or had a foamy appearance. The media of the aorta was loose and edematous; the intima was intact. All other organs (lungs, heart, large arteries, pancreas, spleen, lymph nodes, suprarenal gland, thyroid, parathyroid, brain, hypophysis, intestine, bone marrow) revealed only circulatory changes, such as edema and hemorrhages, or were without any abnormality.

The majority of the organs of the animals subjected to a much more prolonged treatment with gum arabic were normal. In a few dogs the lungs showed small, foam-cellular accumulations in the interstitial tissue and interalveolar septa. The liver was always converted into an organ composed entirely of huge foam cells and often showed pericentral necroses. The foam-cellular transformation also involved the periportal connective tissue as well as the endothelial cells of the hepatic and portal veins (Fig. 1). The spleen presented a similar condition of the pulp, which usually also contained a large number of giant cells of the megakaryocytic type (Fig. 2). The follicles were either normal or showed central hemorrhages. The suprarenal glands contained large masses of foam-cellular reticulum cells in the medulla and not infrequently also in the peripheral cortex (Fig. 3). Foam cells were seen in the reticulum of some of the lymph nodes studied, while others were essentially normal. A moderate number of foam-cellular reticulum cells were present in the bone marrow of one dog. The heart was usually normal. Myocardial arterioles with thickened and hyaline walls were found in one dog. The aortas of all animals showed areas of necrosis and hyalinization of the media, which was also usually calcified, in the region above the aortic valves. The surrounding media was edematous.

The intima above these foci was either normal or consisted of a single layer of small foam cells, or foam-cellular accumulations were present in the subintimal space. A foam-cellular polypous formation of the intima of this region was observed once (Figs. 4 to 6). The carotid and femoral arteries were normal. The renal glomeruli were swollen and had thickened capillary walls which seemed to be embedded in a "mucinous" matrix, sometimes obliterating the glomerular structure to a marked extent. Some of the glomerular tufts were adherent to Bowman's capsules, which were occasionally indistinctly outlined against the surrounding tissue. In some glomeruli there was a marked reduction in the number of nuclei, while in others a focal crowding of oval-shaped nuclei was seen (Figs. 7 and 8). The tubular epithelium was often vacuolated or foam-cellular. Small foam-cellular foci were present in the interstitial tissue in a few cases. The walls of the renal arterioles were usually vacuolated, while a thickened and hyaline media existed in one instance. The brain contained scattered perivascular hemorrhages and masses of foam cells in the choroid plexus. Some of the markedly swollen choroid villi lacked an ependymal covering and were infiltrated with lymphocytes.

II. EGG ALBUMIN: FIRST EXPERIMENT

Egg albumin has a macromolecule which is approximately globular and which has a molecular weight of between 34,500 and 43,800, *i.e.*, it is sufficiently small to pass through the glomerular filter at a relatively rapid rate when introduced intravenously (Bott and Richards; Briggs; Bayliss, Kerridge and Russell). In its hydrated form the molecular weight of this protein does not exceed 61,440 (Bull) and thus remains below the critical limit (68,000) of particle size, which permits glomerular filtration. The studies of Manwaring, Marino, McCleave and Boone have shown that egg white does not readily produce an anaphylactic reaction in dogs, thereby permitting the parenteral introduction of relatively large amounts of this substance without the danger of shock.

The albumin obtained from fresh eggs upon separation from the yolks consists of a thin, watery portion and a thicker, viscous portion containing small, opaque, globular particles. A homogeneous mixture of these two components was made by beating the egg white vigorously until a stiff foam was formed. This was kept in the refrigerator for 24 to 48 hours. A slightly yellow, crystal-clear liquid separated out from the foam. This material had a viscosity of 3.3 to 3.5 at room temperature and a pH of 9.3. The viscosity of the thin and watery portion of the egg albumin identically treated was 3.3 at 20° C. and that of the more viscous portion was 3.42.

The liquid which separated from the beaten whole egg white was injected into the jugular veins of two dogs 5 days a week. The dose given each time was gradually increased from 10 cc. during the first week, to 20 cc. during the next 2 weeks, to 30 cc. during the following 2 weeks, to 40 cc. during the subsequent 3 days, to 50 cc. during the following 5 days, to 65 cc. on the next 2 days, to 80 cc. on the following day, to 90 cc. on the next day, and to 100 cc. on the next 3 days. One dog died directly after the last injection of 65 cc. of egg albumin, while the second dog died immediately after the last of the injections of 100 cc. of egg white. The first dog, weighing 9.06 Kg., received a total of 1060 cc. of egg white, and the second dog, weighing 9.8 Kg., a total of 1330 cc. Several days after the start of the treatment, large amounts of albumin were excreted with the urine, which also contained a few leukocytes and erythrocytes. During the latter part of the experiment the urine contained such large amounts of albumin that upon heating it coagulated to a solid white mass. The head, especially the region of the jaws and eyes, was considerably swollen at times. There were no significant fluctuations in the weights of the two dogs during the 2 months of treatment.

The following tests were made on the blood once a week: amount of hemoglobin, number of erythrocytes and leukocytes, prothrombin time, coagulation time, sedimentation rate, volume of packed erythrocytes, viscosity of whole blood and plasma.

The hemoglobin content remained relatively stationary for the two dogs for the greater part of the experiment and showed a moderate to marked decline only toward the end (12.4 gm. and 14.6 gm., respectively, at the start; 9.5 gm. and 11.6 gm. at the end). Similar fluctuations were observed in regard to the erythrocytes and leukocytes. There was a moderate reduction of erythrocytes and volume of packed blood cells during the second month of the experiment. Prothrombin and coagulation times stayed within normal limits. The sedimentation rate was accelerated to a considerable degree in most tests but returned several times to almost normal values. The viscosity of the whole blood and of the plasma fluctuated within normal limits.

Morphological Pathology

Autopsies of the two dogs showed the spleens to be considerably enlarged, dark red and firm. The livers, kidneys and mucosa of the stomachs and intestines were congested.

Histological examination revealed normal conditions in the lung, pancreas, lymph nodes, heart, adrenal, intestine and stomach. The spleen was considerably congested. The bone marrow was an immature

myeloid tissue. The liver was highly edematous and congested. The liver cell strands were separated by distended sinusoids filled and surrounded by an albuminous material. The liver cells were mildly vacuolated, or small and atrophic. The Kupffer cells frequently contained an amorphous brown pigment. The kidneys of one of the dogs contained numerous wedge-shaped, scarred areas in the cortex composed of round cells, distended tubules with ill-defined epithelial lining and with a stringy, albuminous content, indistinctly outlined multinucleated giant cells in the interstitial fibrous tissue, and arterioles with thickened and hyaline walls and narrowed lumina (Fig. 9). The glomeruli within these areas, as well as in the surrounding tissue, were rich in nuclei and showed thickened capsules. The tubules contained hyaline casts and showed a marked vacuolization and degeneration of the epithelial lining. The kidney of the second dog had distended tubular lumina filled with a stringy, albuminous material and lined by small epithelial cells or by highly vacuolated, swollen cells. The glomeruli were atrophic and small, while the capsular spaces were dilated and filled with an albuminous matter. The walls of the arterioles were swollen, edematous and vacuolated. The renal arteries in both dogs were normal.

The ascending aorta of one dog showed a large hyaline area with necrosis and central calcification, situated in the outer media. There were focal granular and diffuse calcific deposits in the inner media within subintimal, hyaline scar tissue in the ascending aorta of the second dog (Fig. 10). The endothelium of this aorta had proliferated and showed palisade arrangement elsewhere. The coronary arteries had highly edematous walls and showed distortion of their normal structure. A small hyaline intimal thickening was found in one of the small abdominal arteries (Fig. 11).

EGG ALBUMIN: SECOND EXPERIMENT

In a second experiment, egg albumin was combined by diazotization, using the method of Landsteiner, with amino-antipyrine, producing thereby an amino-antipyrine-azoprotein. The original purpose of this experiment was the experimental production of an agranulocytosis on the basis of a specific allergy, through sensitization of dogs by repeated intravenous injections of a 1 per cent solution of this azoprotein. While this goal was not achieved, significant vascular changes were observed.

Two dogs, both weighing approximately 10 Kg., were used. They received 10 cc. of a 1 per cent solution of amino-antipyrine-azoprotein into the jugular vein five times a week. After 21 injections had been given within 3 weeks, the treatment was discontinued for 2 days, and 0.32 gm. of amino-antipyrine was given by mouth on 4 successive days.

The intravenous medication was continued during the next 2 days and then stopped. Thus, a total of 230 cc. of 1 per cent amino-antipyrine-azoprotein was administered. Because the dogs showed signs of intoxication, no treatment of any kind was given for 1 week. Subsequently, for a period of 3 months, the dogs received on alternate days, 0.32 gm. of amino-antipyrine orally. As there was no appreciable effect upon the status of the leukocytes, no further treatment was given; the dogs were killed approximately 2 months after the last medication of amino-antipyrine.

Morphological Pathology

The autopsies showed small kidneys with numerous cicatricial cortical retractions. The spleens were small, dark red and had granular surfaces.

Upon histological examination the walls of some of the myocardial arterioles were found to be in part bluish-stained; others had thickened, hyaline walls lined by a proliferated and clumped endothelium. The majority of the coronary arteries, however, were normal. The myocardium was without any abnormalities. The liver cells had a fine vacuolar or foamy cytoplasm. The adrenals were normal. The spleen was congested. Some amorphous brown pigment was located within the cytoplasm of large, reticular foam cells, which were found in focal accumulations or scattered singly throughout the splenic pulp. Their cytoplasm stained a grayish blue. The spermatogenic epithelium of the testes was highly atrophic. The bone marrow of the sternum was a myeloid fat tissue containing numerous mature cells.

The kidneys showed in the cortex localized mononuclear and foam-cellular infiltrations and wedge-shaped areas of fibrous scar tissue. The afferent arterioles and the pedicles of the glomeruli often had thickened hyaline or very cellular walls. The capillary loops were frequently thickened and hyaline, converting the glomerulus into an irregular, coarse network of hyaline bands in which irregularly shaped nuclei were found. The capillary lumina of such glomeruli were distended and sometimes cystic. In others there was an almost complete obliteration of the capillary structure by the swollen and coalescing, hyaline, vascular structures. In some of the cystic glomerular cavities foam cells were found or large, syncytial, bluish-stained masses with numerous, clumped, pale-stained nuclei (Fig. 12). The hyalinizing processes sometimes involved only a part of a glomerulus. The walls of some of the medium-sized arteries were highly vacuolated, while the walls of the arterioles showed a moderate to marked hyaline thickening with swelling and foam-cellular transformation of the endothelium leading

to an almost complete occlusion of the lumina. The tubular epithelium was essentially normal.

The intima of the aorta occasionally contained small foam cells, which invaded the superficial layers of the underlying edematous media (Fig. 13). In other parts there were a few large hyaline scars in the media of the aorta. In sections of some of the large elastic arteries there was found an extreme cushionlike, fibrous thickening of the intima. Groups of small foam cells were scattered through these fibrous and partly hyalinized thickenings and were situated mainly near the media (Fig. 14). Smaller cushions consisted almost exclusively of foam-cellular proliferations of the intima.

III. GELATIN

Gelatin, the water-soluble derivative of collagen, varies considerably in its molecular weight (35,000 to 150,000), depending upon the method of preparation. It is for this reason that only those portions and types of gelatin having a molecular weight below 68,000 when hydrated will pass through the glomerular filter, while the larger molecular types of gelatin are retained at least temporarily in the body after parenteral introduction. While impure gelatin gives rise to the development of allergic and toxic reactions (Taylor and Waters), it is usually contended that purified gelatin does not possess allergenic properties, because cyclic amino acids are absent in its molecule.

A high-grade gelatin (Coignet Père and Fils, Silver Label) was dissolved over a steam bath in a 1 per cent sodium chloride solution. The hot solution was mixed with a large amount of "Hyflo-Supercel" and filtered through Whatman no. 1 filter paper in a Büchner funnel, using strong suction. The filtered solution, which was brown and slightly cloudy, was then autoclaved at 15 lbs. for 20 minutes.

A 1 per cent solution thus prepared has, at 24° C., a viscosity of 1.38; a 2 per cent solution has a viscosity of 1.88, or approximately that of normal human and canine plasma. A 17 per cent solution has a pH of 5.25.

Five dogs were employed in the experiment. Four of them were injected intravenously, while the fifth dog, for the greater part of the experiment, was injected intraperitoneally after it became difficult, following 6 intravenous injections, to enter the jugular vein because of the extremely short neck of this animal. The weight of the dogs ranged from 5.6 to 12.37 Kg. The injections were given five times weekly. The concentration as well as the dose of gelatin solution administered was gradually increased. After 2 injections of 10 cc. of a 10 per cent solution, 3 injections of 10 cc. of a 17 per cent solution

were given. The dose was then increased to 15 cc., 20 cc. and 40 cc., on successive days. After 3 injections of 40 cc. the dose was increased for each of the following 2 injections to 60 cc. and 65 cc. After 1 injection of 80 cc. and 1 of 100 cc. the daily dose was elevated to 120 cc. for the next 5 injections. For the subsequent 4 administrations of 120 cc. the concentration of the gelatin solution was increased from 17 per cent to 25 per cent. No further treatment was given to two dogs which were killed 4 weeks after the last injection. In two other dogs of this series the treatment was resumed after 4 weeks for a period of 6 days, during which 120 cc. of a 25 per cent solution was injected each day. Two days after the last injection these two dogs were killed. The fifth dog in this series was killed after it had received 2 injections of 10 cc. each of a 10 per cent solution, 3 injections of 10 cc. and 1 injection of 15 cc. of a 17 per cent solution, as it showed signs of severe dyspnea. This dog received within 8 days, during 5 injections, 65 cc. of gelatin solution containing a total of 9.65 gm. of dry gelatin. Two dogs received within 2 months, 1840 cc. of gelatin solution containing 414 gm. of gelatin in 27 injections, and two other dogs, in 39 injections, received 2,849 cc. of gelatin solution containing a total of 576 gm. of gelatin within 11 weeks.

Apart from a somewhat increased salivation observed directly after the injection of the larger doses of gelatin solution, there were no symptomatic reactions of any kind. The urinalysis made at irregular intervals showed only traces of albumin and an occasional leukocyte and erythrocyte in the sediment.

The same tests of the blood as in the study of the effect of egg albumin were made at weekly intervals. The amount of hemoglobin, the number of erythrocytes and the volume of packed red blood cells decreased considerably with the progressive injection of gelatin solution, but underwent a relatively rapid recovery after cessation of treatment. At the same time the sedimentation rate increased rapidly and considerably. The leukocytes, prothrombin time and coagulation time fluctuated markedly but remained within the extremes of the normal range. The viscosity of the whole blood and especially that of the plasma became appreciably increased beyond the normal limit, particularly during the final part of the experiment, when large doses of a 25 per cent gelatin solution were given. Table IV, giving the data obtained in dog no. 724, weighing 5.6 Kg., is a representative example of the observations made.

In view of the fact that the increased viscosity of the blood and plasma indicated the retention of gelatin in the blood, an attempt was made to demonstrate the presence of this material by an indirect

method. Twenty-four hours after the dogs had received five injections, during 5 days, of 120 cc. of the 25 per cent gelatin solution, blood was withdrawn and mixed with sufficient amounts of potassium oxalate to prevent clotting. This blood solidified when placed in the refrigerator and became liquid again when heated to body temperature. Plasma from a normal dog and from the animals injected with gelatin was

TABLE IV

Blood Reactions during Repeated and Massive Intravenous Administration of Gelatin Solution

Time	Gelatin	Hemoglobin	Erythrocytes	Volume packed erythrocytes	Sedimentation rate	Leukocytes	Viscosity	
							Whole blood	Plasma
	(gm.)			(mm.)	(mm.)			
1st day		13.8	6,630,000	44.5	1.0	15,100	4.76 at 22.2° C.	1.93 at 22.5°C.
6th day	2.0	12.5	5,910,000	41.0	32.0	20,300	5.48 at 19.2°	2.16 at 21.0°
16th day	23.25	12.1	6,270,000	41.0	45.0	25,800	5.06 at 19.0	2.05 at 21.1°
22nd day	41.95	10.5	5,010,000	36.0	55.0	16,500	4.10 at 21.8°	2.30 at 21.8°
30th day	77.65	11.8	6,000,000	35.0	58.0	14,500	4.76 at 23.4°	2.35 at 19.4°
36th day	108.25	10.3	5,060,000	32.0	61.0	15,100	4.66 at 21.5°	2.36 at 19.6°
43rd day	150.75	10.9	5,690,000	29.5	58.0	16,100	5.70 at 20.0°	2.72 at 18.5°
51st day	253.60	8.0	3,770,000	28.0	58.0	15,300	8.10 at 22.3°	4.3 at 19.3°
57th day	324.40	8.3	4,480,000	29.0	69.0	20,500	6.04 at 22.0°	3.80 at 21.1°
63rd day	414.40	5.9	3,500,000	23.0	62.0	14,000	4.10 at 21.4°	2.68 at 20.0°

then heated in test tubes in a water bath to a temperature of 95° C. for 5 minutes, when the proteins were well coagulated. The coagulum was then broken up by a wire, and the tubes, while hot, were centrifuged for 30 minutes. About 1.5 cc. of a liquid material was expressed thereby from an original amount of 3 cc. of plasma. This expressed liquid was clear and pale straw-colored. When the fluid expressed from the coagulated normal plasma was placed in the refrigerator, it remained liquid, while that obtained from the plasma of the animals injected with gelatin solidified, showing thereby that it was composed in part of gelatin.

Morphological Pathology

Autopsies of the dogs showed essentially normal organs upon gross examination. The spleens were in general moderately enlarged, dark red and semifirm.

Histological examination showed that the lungs, pituitary body,

brain, pancreas, spleen, lymph nodes, bone marrow and intestines were without abnormalities. The liver was edematous and contained moderately atrophic or vacuolated cells. The myocardium was normal, while the coronary arterioles showed swollen, vacuolated and edematous walls with focal hyalinization in some instances (Fig. 15). The aorta and the large and medium-sized arteries of the abdomen revealed moderate to marked medial edema in two dogs. In addition to similar edematous changes there were subintimal and medial calcification and hyalinization in the arteries of the other three dogs of this series. The intimal hyaline and fibrous thickenings were very extensive in the aorta of the dog which was injected intraperitoneally (Fig. 16). The calcification found in this case involved not only areas of medial necrosis but also the internal elastic membrane (Fig. 17). A smaller artery showed a dissecting aneurysm destroying the media in large areas. While the kidneys of two dogs were essentially normal, in the other three dogs these organs showed a marked vacuolization of the tubular epithelium. The walls of the renal arteries and arterioles were vacuolated and edematous, and medial hyalinization and intimal hyaline thickenings were found in some instances. Some of the arterioles were occluded by hyaline masses infiltrating the walls and containing irregularly arranged and shaped nuclei (Fig. 18). Small polypous endothelial proliferations were seen in the branches of the renal artery. The glomerular capillaries in the dog injected intraperitoneally were distended and showed a marked, sometimes networklike, proliferation of the endothelial cells. The glomeruli were swollen and congested and erythrocytes were present in some of the Bowman's capsular spaces of this dog.

Comment

The observations presented show that the intravenous injection of solutions of gum arabic, ovalbumin and gelatin elicit in the blood a complex of cellular and plasmatic reactions characteristic of macromolecular hematic disturbances and similar to those caused by the intravenous introduction of polyvinyl alcohol, methyl cellulose and pectin; *i.e.*, substances used or proposed as nonhematogenous blood substitutes. The data recorded indicate that the protracted phase of this response, following upon the repeated administration of the substances studied, is in general less pronounced than that seen with polyvinyl alcohol and methyl cellulose and resembles more closely that found with pectin.

It is remarkable that even after a very intensive treatment with large amounts of highly viscous solutions of gum arabic and gelatin,

there develops only a relatively minor increase in the viscosity of the plasma, an effect which contrasts sharply with the marked and sustained elevations in plasmatic viscosity obtained by the injection of solutions of polyvinyl alcohol and methyl cellulose. The gelation reaction of the plasma in animals injected with gelatin as well as the physicochemical plasmatic disturbances and the presence of a massive granulation of extraneous matter in the plasma of the dogs injected with gum arabic are, on the other hand, satisfactory proof that the blood contains considerable amounts of the injected foreign material. These findings suggest that the macromolecules of these substances undergo in the blood a certain amount of degradation resulting in a reduction of the viscosity of their colloidal solution. This observation is important for therapeutic reasons, as the size of the molecule controls the degree of retention of these substances in the blood and thus also the magnitude and duration of the colloidal osmotic pressure exerted by them.

The behavior of gum arabic and gelatin is explainable by the fact that gum arabic is relatively unstable and tends to become partially hydrolyzed, while gelatin is readily degraded by the proteolytic enzymes present in the body into its amino acid constituents, which are used in the normal metabolism. It is apparently for these reasons that gelatin, parenterally introduced, does not give rise to appreciable phenomena, which form a typical and remarkable feature of the organic lesions elicited by gum arabic. Both substances, however, seem to remain sufficiently long in a macromolecular state in the blood to cause degenerative changes in the glomerular filtration membranes.

Somewhat different conditions apparently prevail in these respects in regard to the injected egg albumin. The observations reported by Briggs, as well as the renal and urinary findings recorded here, indicate that a large portion of the ovalbumin intravenously introduced is rapidly excreted through the kidneys. However, only a part of the albumin demonstrable in the urine under such conditions represents egg albumin, while another portion is serum albumin to which the glomerular filter becomes permeable following the passage of the foreign protein (Briggs). Moreover, this investigator pointed out that a part of the injected ovalbumin remains in the organism and is present in the blood over prolonged periods. The glomerular lesions found in the dogs treated with this protein suggest that ovalbumin or complexes formed by it may clog the glomerular capillaries and lead to a distension of their lumina, to the development of hyaline changes and to a proliferation of endothelial cells and giant cells (Welker, Andrews and Thomas). It is noteworthy that the renal lesions were particularly severe in the dogs injected with the allergenic azoprotein.

In view of the thesis advanced concerning the causal relationship between hematic colloidal disturbances and arteriosclerotic or atheromatous lesions, it is significant that the physicochemical macromolecular changes produced in the plasma of the animals treated with gum arabic, gelatin and ovalbumin are accompanied by the appearance of degenerative and proliferative reactions in arterial walls. It is noteworthy that the arterial and arteriolar lesions present in the animals injected with the two foreign proteins are predominantly of the sclerosing type; *i.e.*, they consist of marked fibrous intimal thickening and medial hyalinization and necrosis and are not of the atheromatous character.

This response of the arterial, and especially the intimal, tissues to the presence of foreign proteins and their metabolic or allergic reaction products seems to be in accord with the chemical nature of these substances, as their inclusion into the cytoplasmic matter of the reacting intimal cells cannot lead to the primary formation of foam cells. Such an event under existing experimental conditions is either the result of a secondary cellular degeneration or represents the product of an excessive cytoplasmic hydration.

The arterial lesions elicited in these dogs are comparable in type and causative mechanism to the arteriosclerotic changes seen by Dick and Leiter in rabbits in connection with the hyperglobulinemia and amyloidosis following the repeated injection of streptococcic cultures. They are also similar to the arterial lesions described by Holman in dogs subjected to repeated intravenous injection of large amounts of altered plasma and attributed to an allergic reaction. However, they differ in morphological type, as well as in chemical nature of the causal substances involved, from the arterial lesions elicited in rabbits by disturbances of protein metabolism, as rabbits kept on a diet rich in proteins develop a hypercholesterolemia and cholesterol atheromatosis (Clarkson and Newburgh; Meeker and Kesten).

The foam-cellular, atheromatous changes observed in the animals treated with gum arabic represent, on the other hand, counterparts to the lesions elicited by hypercholesterolemia, polyvinylosis and methyl cellulosis. It appears likely that the relatively moderate degree of these changes in animals treated with gum arabic, when compared with those in animals treated with cholesterol, polyvinyl alcohol or methyl cellulose, is attributable to the instability of the macromolecules of gum arabic and the resulting fleeting nature of the disturbances in the colloidal plasmatic equilibrium.

It is probable, on the other hand, that the mechanism in the production of the arterial lesions in chronic arabinosis is identical with

that in atheromatosis associated with cholesterolosis, polyvinylosis and methyl cellulosis. Christie, Phatak and Olney have demonstrated that gum arabic solution injected intravenously interferes with the oxygenation of erythrocytes. Previous studies (Martin and Hueper) have shown that the blood of dogs given polyvinyl alcohol and of rabbits given cholesterol exhibits a similar impairment of the oxygenation speed of the erythrocytes, probably by the formation of a film of these substances on the surface of the cells. It is postulated that a similar film on the surface of the intima of vessels exerts a corresponding effect upon the exchange of essential metabolites and thus produces a defective oxygenation and nutrition of the arterial walls. In view of the fact that the cholesterol level of the blood is lowered in animals injected with gum arabic, it is unlikely that cholesterol plays any significant part in the causation of the arterial changes.

Leary recently contended that cholesterol atheromatosis in man and rabbits is caused by the active invasion of the arterial walls by lipoidal, foam-cellular phagocytes circulating in the blood and originating from desquamated Kupffer cells and reticulum cells of the adrenal. These cells are said to take up excess cholesterol esters, to possess an extraordinary stickiness after their escape into the blood stream and to have a special, although unexplained, affinity for the tissues of the vascular wall, where they lead a prolonged existence and stimulate the growth of connective tissue.

The present observations with gum arabic indicate that in spite of an extensive transformation of the reticulo-endothelial cells of the liver and adrenal and of other histiocytes into foam-cellular elements, there occurred only moderate atheromatous formation. While attempts at demonstrating foam cells in the numerous blood smears taken during the course of the experiment were unsuccessful, it cannot justly be assumed that these reticulo-endothelial cells, swollen with gum arabic, do not desquamate and do not circulate in the blood. Histological studies did not succeed in demonstrating in a single instance in these and previous experiments the presence of a thrombuslike attachment of foam cells to the intima of any vessel. Moreover, it appears unlikely that such huge foam cells should stick to a much higher degree to the intimal surface of large vessels with their swift blood current than to the wall of small vessels and capillaries, in the lumina of which these cells become temporarily or permanently arrested. There was, on the other hand, a direct foam-cellular transformation of the intimal lining of the aorta, similar to that seen frequently in the vessels of various species treated with polyvinyl alcohol, methyl cellulose or cholesterol. It may be doubted, furthermore, that reticulo-endothelial cells loaded

to capacity with foreign matter and separated from their normal connection possess such extraordinarily aggressive qualities and prolonged survival rate. It is known that cells of similar derivation, such as megakaryocytes, released into the blood under abnormal conditions, undergo rather rapid disintegration in the lumina of the pulmonary capillaries.

It is, moreover, a pure assumption that lipophagic foam cells possess a special attraction to the arterial wall, and that for this reason the resulting atheroma represents the normal graveyard of these cells. There can be no doubt that foam cells containing other substances (polyvinyl alcohol, methyl cellulose, pectin, gum arabic) do not exhibit such an affinity. Yet huge, foam-cellular mononuclear cells can be readily demonstrated in the blood smears of dogs treated with methyl cellulose.

Conclusions

The intravenous injection of colloidal solutions of gum arabic, gelatin and ovalbumin elicits in the blood of dogs and rabbits responses which are characteristic of the "macromolecular hematological syndrome" (reduction of erythrocytes and hemoglobin, hastened sedimentation of erythrocytes, transitory leukopenia, persistent leukocytosis or leukopenia and reduction of serum proteins) and represent, in part, expression of the disturbances produced in the equilibrium of the colloidally dispersed macromolecules of the plasma.

The development of storage phenomena in the internal organs depends upon the stability of the injected macromolecules. The injection of gelatin and ovalbumin therefore does not give rise to a thesaurosis, while gum arabic, which cannot be readily and completely depolymerized in the body, is retained in the liver cells and in the reticuloendothelial cells and histiocytes of this organ and of the kidney, spleen, lymph nodes and adrenal.

All three substances apparently cause a clogging of the glomerular filtration membranes when present in excessive amounts in the blood, and thereby elicit degenerative and proliferative changes in the glomeruli.

The injected foreign proteins produce degenerative and proliferative lesions in the elastic and muscular arteries which are of the sclerosing type, while gum arabic causes the development of atheromatous responses. The chemical type and the physicochemical properties of the causative macromolecular agent thus determine the morphological character of the ensuing arterial reactions.

The agents, such as macromolecular carbohydrates (polyvinyl alco-

hol, pectin, methyl cellulose, gum arabic) and lipoids (cholesterol), which form emulsions with proteinic solutions, give rise to foam-cellular atheromatous lesions.

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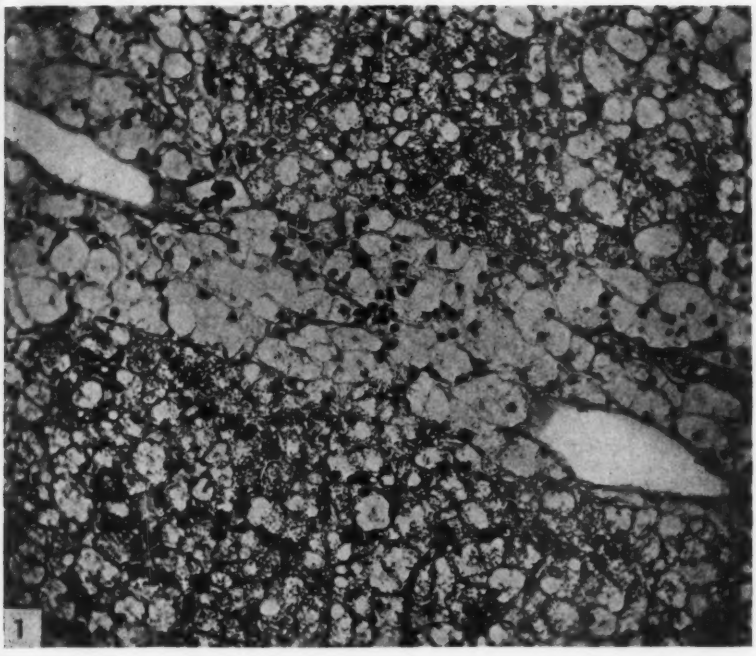
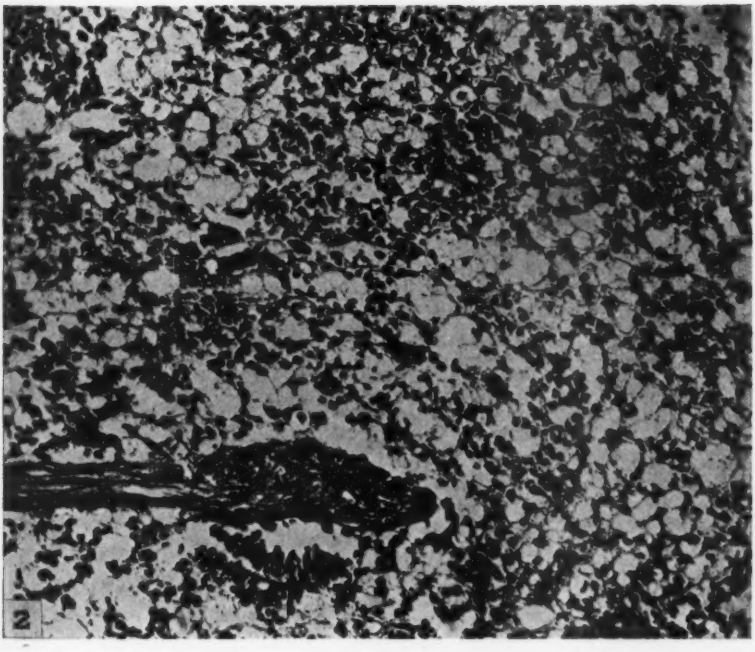
DESCRIPTION OF PLATES

PLATE 133

FIG. 1. Hepatic veins lined in part by foam cells and surrounded by a strand of light-colored connective tissue foam cells, on both sides of which foam-cellular liver cells are found. × 230.

FIG. 2. Foam-cellular splenic pulp with several megakaryocytic giant cells. × 230.



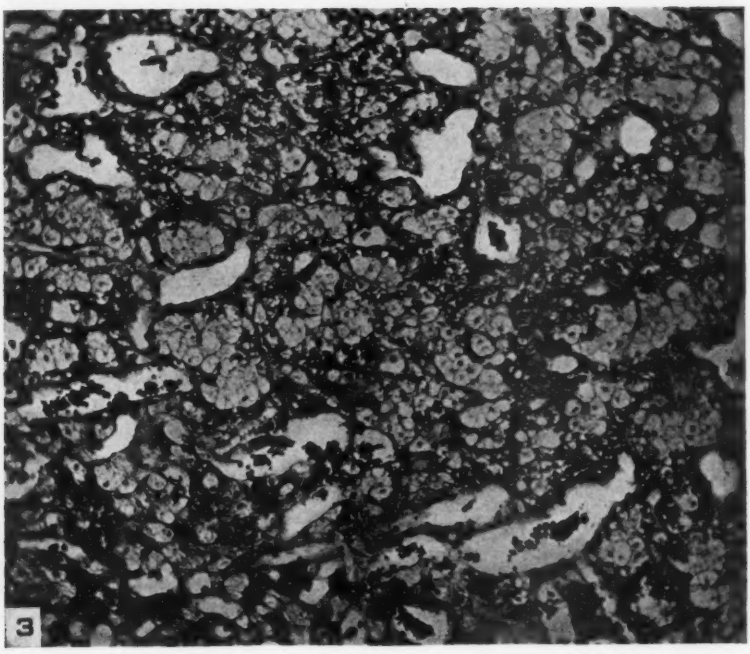


Hueper Intravenous Injections of Macromolecular Substances

PLATE 134

FIG. 3. Adrenal medulla with diffuse foam-cellular transformation of the reticulum cells. × 110.

FIG. 4. Foam-cellular transformation of the intima of the aorta with foam-cellular infiltration of the inner parts of the media. × 230.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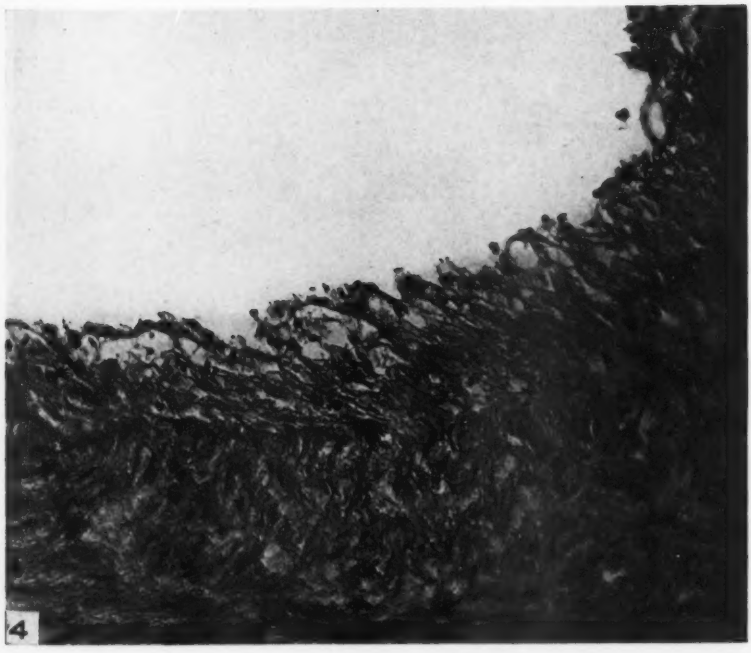
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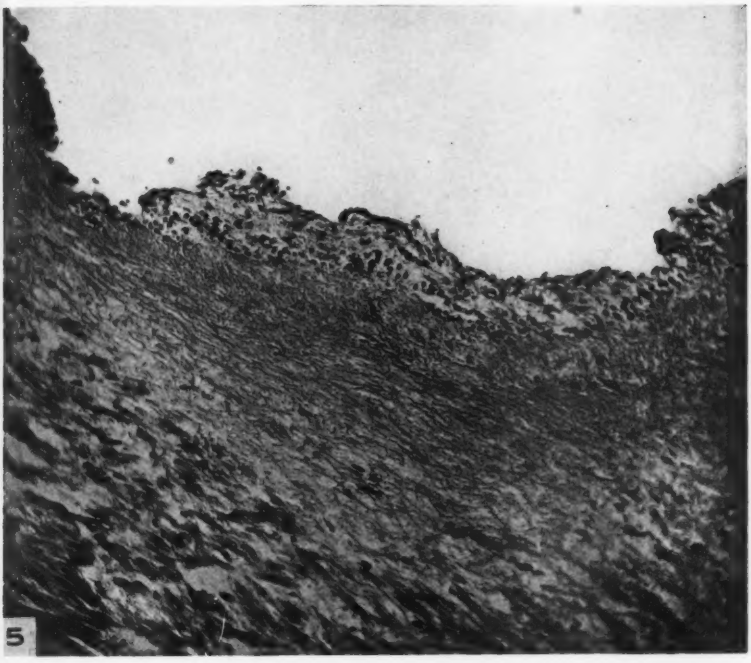
PLATE 135

FIG. 5. Foam-cellular intimal thickening of the aorta with an underlying necrosis of the media. × 150.

FIG. 6. Large calcified necrosis in the media of the aorta with a small foam-cellular accumulation in the subintima. × 120.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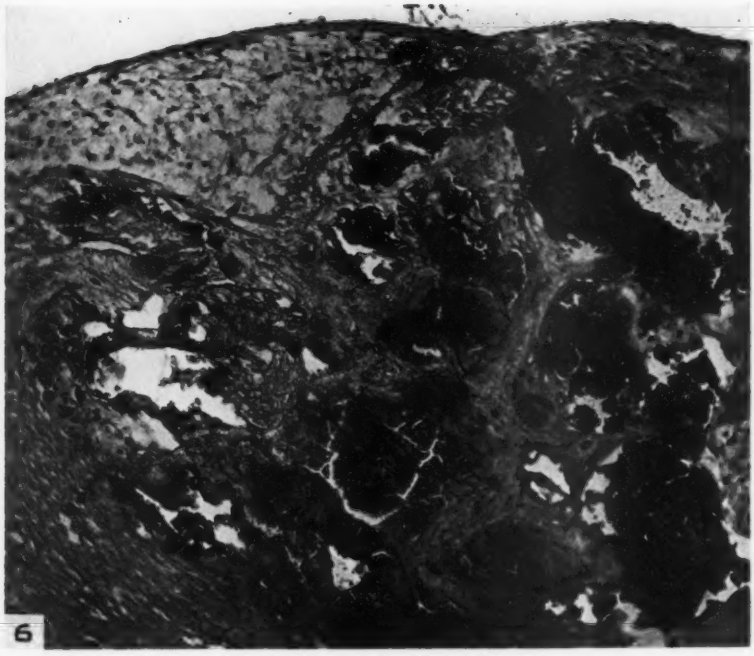
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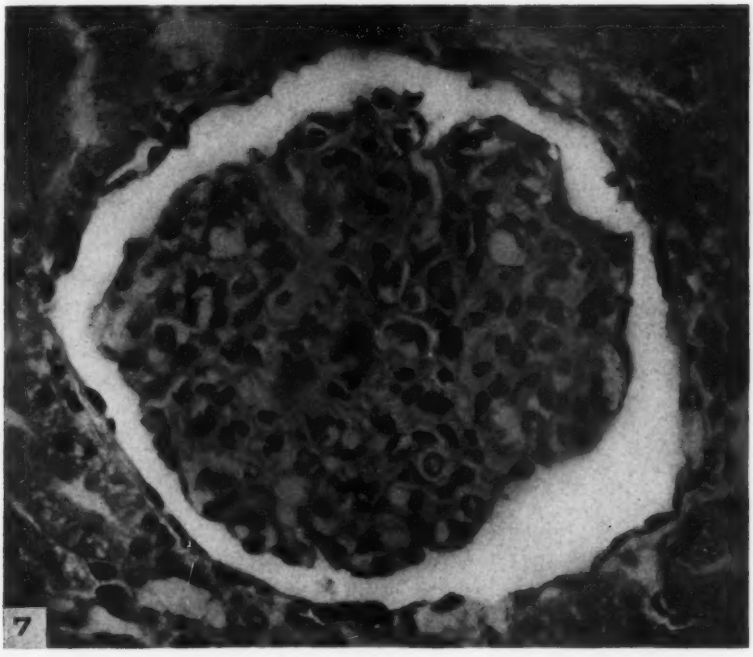
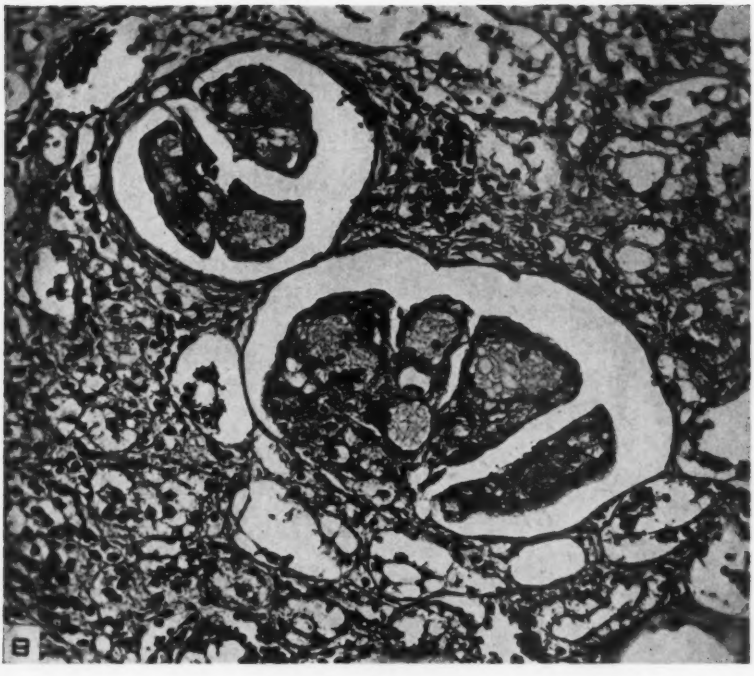
PLATE 136

FIG. 7. Glomerulus in the kidney of a dog with (mucohyaline) thickening of the glomerular framework and a proliferation of the endothelial cells. × 425.

FIG. 8. Glomeruli in the kidney of a rabbit with multicystic swelling of the capillary loops and accumulation of foam cells in the glomeruli and in the surrounding interstitial tissue. × 260.



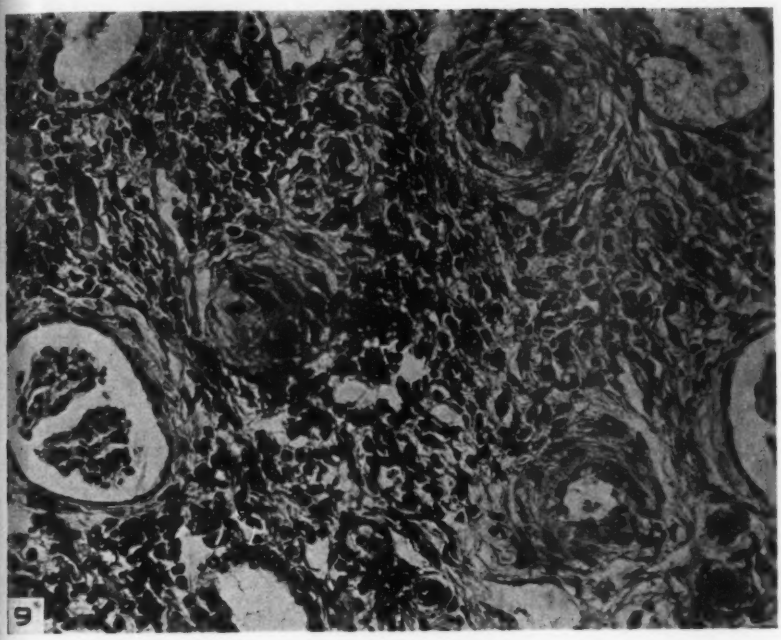


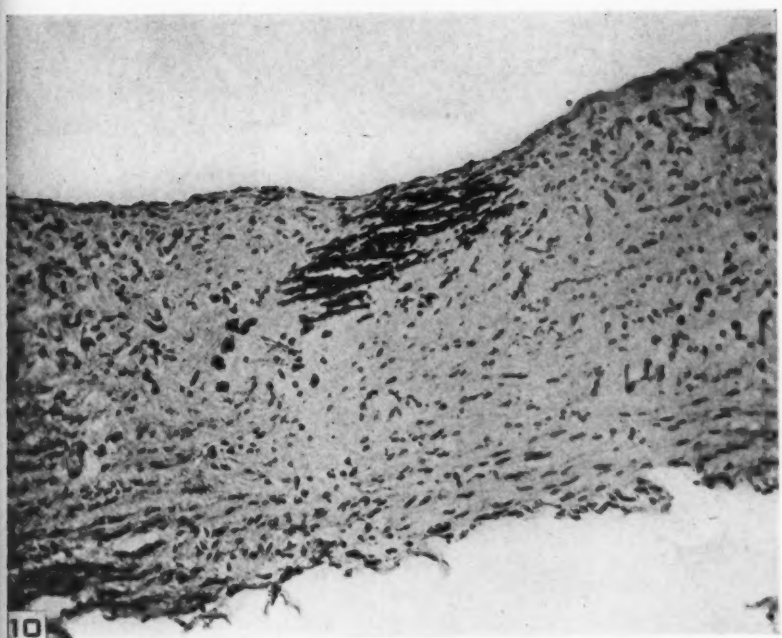
Hueper Intravenous Injections of Macromolecular Substances

PLATE 137

FIG. 9. Renal arterioles with thickened walls and proliferated endothelium embedded in a fibrous stroma infiltrated with lymphocytes. × 230.

FIG. 10. Wall of ascending aorta with hyaline medial necrosis showing local calcification. × 230.



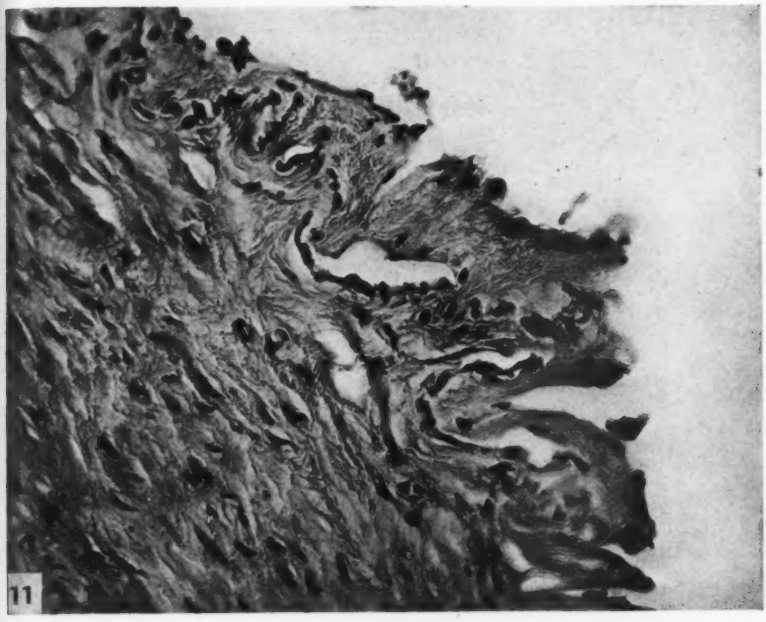


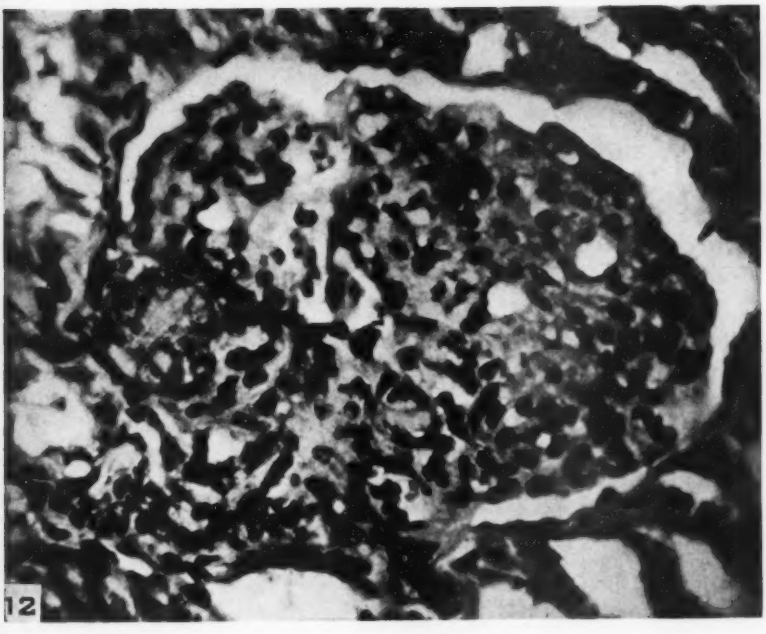
Harper Intravenous Injections of Macromolecular Substances

PLATE 138

FIG. 11. Small abdominal branch of aorta with localized hyaline intimal thickening. × 350.

FIG. 12. Glomerulus with multinucleated, syncytial mass and hydropic swelling of the capillary network. × 425.



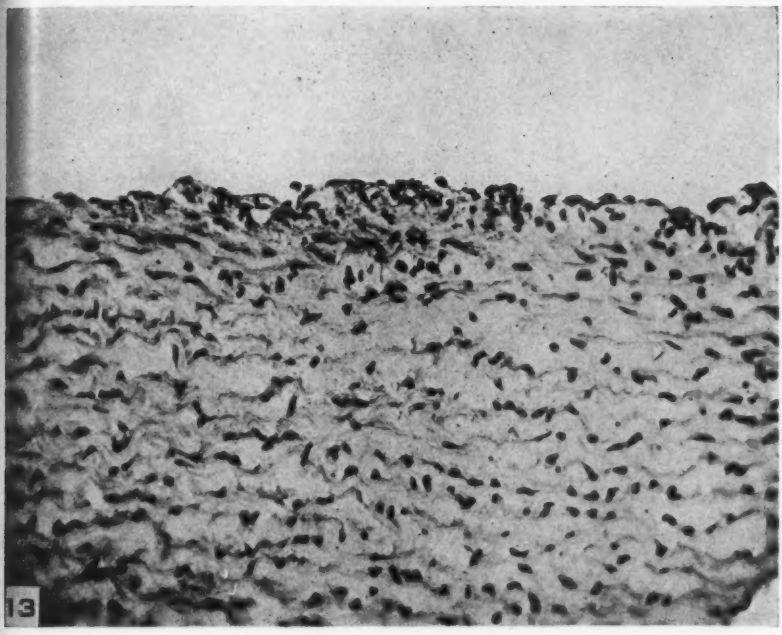


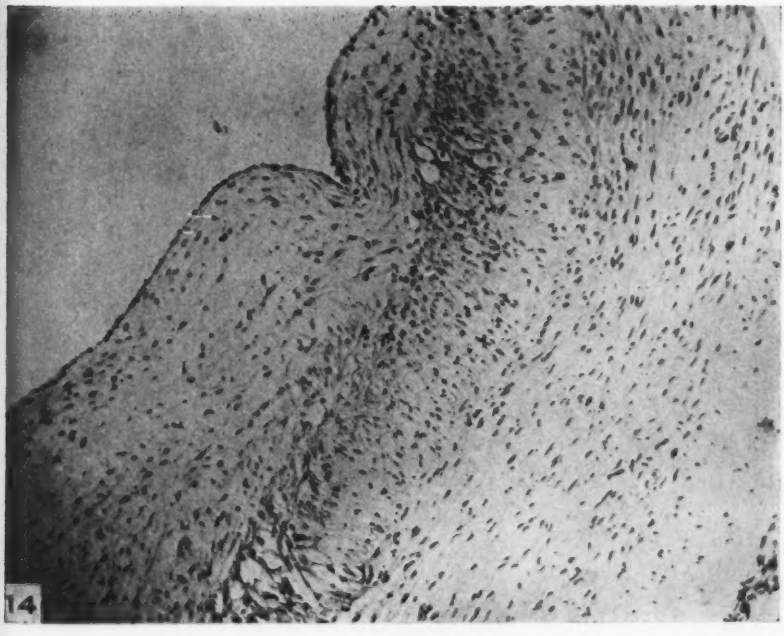
Hueper Intravenous Injections of Macromolecular Substances

PLATE 139

FIG. 13. Edematous wall of aorta with hydropic mononuclear cells underneath the intima. × 230.

FIG. 14. Thick cushionlike fibrous intimal thickening of aorta with foam-cellular elements in its basal portion. × 125.





Hueper Intravenous Injections of Macromolecular Substances

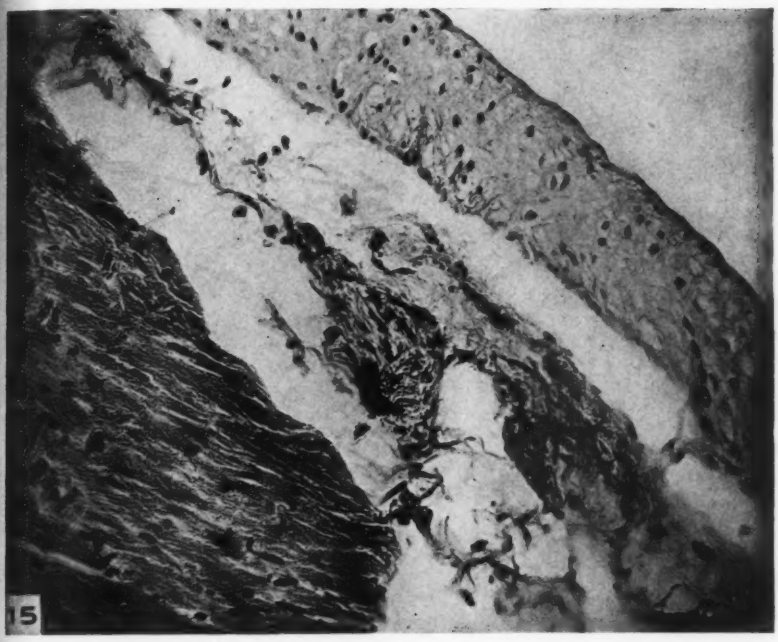
PLATE 140

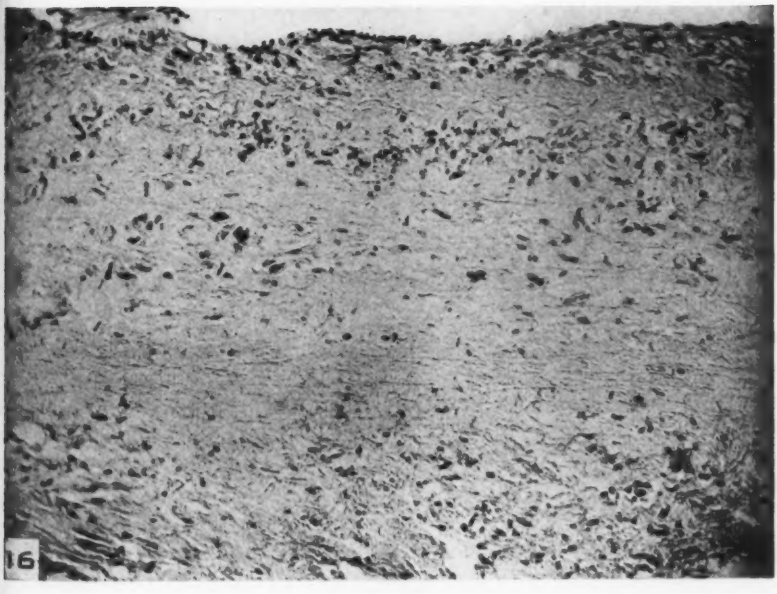
FIG. 15. Myocardial artery with partly hyalinized and vacuolated media. × 175.

FIG. 16. Extensive medial hyalinization in the aorta. × 110.

Автокс

Пере



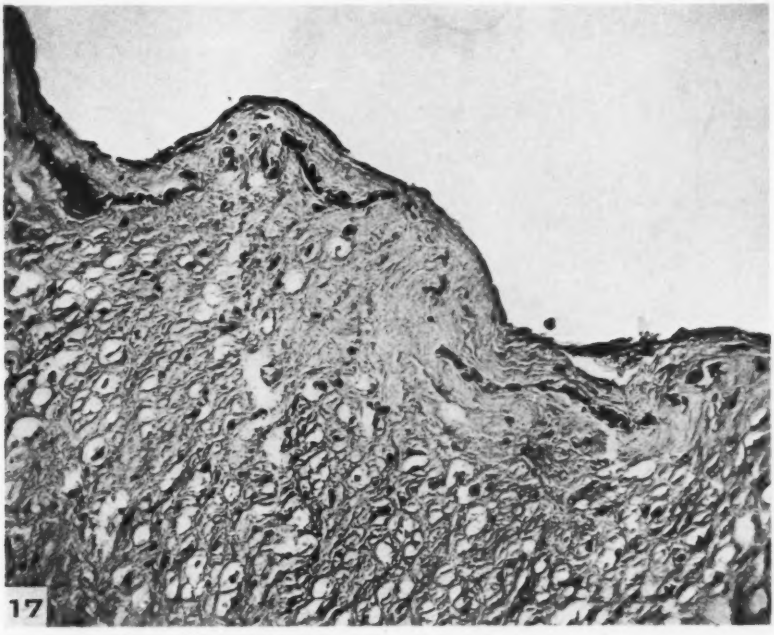


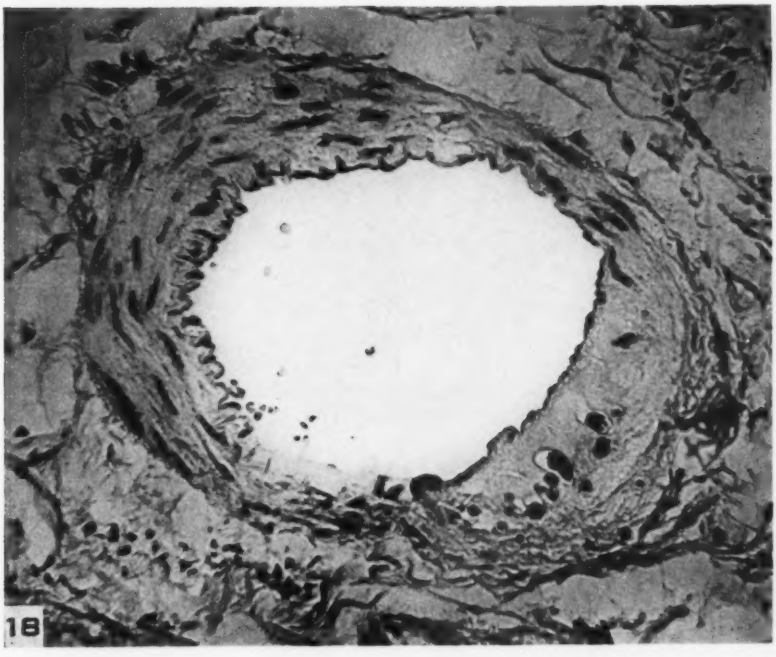
Hueper Intravenous Injections of Macromolecular Substances

PLATE 141

FIG. 17. Intimal hyaline thickening of aorta with calcification of internal elastic membrane. × 150.

FIG. 18. Renal arteriole with hyalinization and swelling of wall. × 230.





Hueper Intravenous Injections of Macromolecular Substances

SPONTANEOUS CEREBELLAR HEMORRHAGE*

Report of Fifteen Cases

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(From the Department of Pathology, Queens General Hospital, Jamaica, N.Y.)

Beginning with the 3 cases described by Morgagni and Lieutard and cited by Sédillot in 1813,[1] there has been a total of 109 cases of non-traumatic cerebellar hemorrhage reported in the literature. This group of cases will be designated as *series A*. From November, 1935 to November, 1941, 3,881 autopsies were performed in the Department of Pathology at Queens General Hospital. During this period, a total of 115 spontaneous intracranial hemorrhages were observed. Of these, 100 were located in the cerebrum and/or brain stem, while 15 were found in the cerebellum proper. The latter 15 instances will be referred to hereafter as *series B*. All cases of cerebral thrombosis with softening, old hemorrhages of the cerebrum and the cerebellum and traumatic intracerebral and intracerebellar hemorrhage are excluded from consideration.

Reports of Cases

Case 1

F. T. (A-40-266M), a white female, 65 years old, suddenly became comatose 8 hours before admission. For a few months purpuric spots had been noted in the skin associated with firm subcutaneous masses in the thighs and breasts. Physical examination revealed an acutely ill white female in deep coma. The deep reflexes and blood pressure were unobtainable. She died 1 hour after admission. Autopsy revealed a massive recent hemorrhage in the right lobe of the cerebellum occupying four-fifths of the white matter with extension to the vermis and the regional cortex (Fig. 1). The ventricles were filled with fluid blood. In addition, there were small focal hemorrhages in the corpus callosum and midbrain. Examination of the viscera revealed extensive leukemic infiltration of the bone marrow, spleen, liver, left breast, subcutaneous tissue of both thighs and antecubital fossae, trachea, heart, stomach (with massive ulceration), renal medullary pyramids and renal cortex. Microscopy confirmed extensive leukemic infiltration in the sites mentioned and in the region of the cerebellar hemorrhage (Figs. 2 and 3). The final diagnosis was an acute exacerbation of a chronic myelogenous leukemia with fatal terminal cerebellar hemorrhage.

Case 2

J. S. (A-39-256), a white female, 5 years old, entered the hospital because of weakness, loss of weight and purpura. Hematological studies established the presence of a chronic myelogenous leukemia with an acute exacerbation. Respiratory distress was noted terminally after a prolonged downhill course. In addition to widespread leukemic infiltration of all lymph node structures and most of the viscera, an area of recent hemorrhage, 4 by 2½ cm., was found in the left cerebellar hemisphere in the region of the dentate nucleus near the vermis (Fig. 4). The

* Received for publication, January 22, 1942.

hemorrhage had extended through the ependyma of the fourth ventricle with a complete blood clot cast of all the ventricles. Microscopically, large numbers of immature myeloid cells were seen intimately associated with the hemorrhage.

Case 3

E. R. (A-40-154M), a white male, 74 years old, suddenly complained of a violent headache. He became comatose within a short interval and died several minutes after admission to the hospital. Autopsy revealed a massive hemorrhage almost completely filling the right cerebellar hemisphere, with extension to the vermis, and subependymal hemorrhages beneath the fourth ventricle. The ventricular cavities contained no blood. Regional subarachnoid hemorrhage of moderate degree was present. Secondary streaked hemorrhages were seen in the pons. The heart was moderately hypertrophied, weighing 500 gm.

Case 4

M. W. (A-39-84), a white female, 65 years old, suddenly became dizzy and nauseated while riding on the subway. On admission to the hospital, she complained of severe headache. Definite nystagmus was present with the slow component to the right. The systolic blood pressure was over 300 mm. Spinal tap yielded bloody fluid, under markedly increased pressure. She expired after 6 hours. The immediate cause of death was a massive recent hemorrhage completely destroying the dentate nucleus and regional white matter of the left lobe of the cerebellum (Fig. 5). All ventricles were filled with blood clot and fluid blood. In addition, an old area of softening was found in the right basal ganglia, with limited hemorrhage. Marked cerebral atherosclerosis, arteriolar nephrosclerosis and hypertrophy of the left ventricle were also noted.

Case 5

S. S. (A-39-227M), an elderly white male, died suddenly with no previous history available. Autopsy findings consisted of massive cerebellar hemorrhage, arteriolar nephrosclerosis, moderate hypertrophy of the heart (420 gm.) and marked cerebral atherosclerosis. In the right cerebellar hemisphere there was noted a large cavity 12 cm. in diameter, completely filled with dark red clotted blood. There was limited extension to the left lobe. All ventricles were occupied by dark red, fluid and clotted blood. The cisterna magna contained approximately 4 cc. of blood.

Case 6

C. K. (X-39-202), a white male, 63 years old, had known that he suffered from heart disease and diabetes for 2 years. On the morning of death, he noted the sudden onset of "terrific" headache and dizziness. He died within 1 hour, before medical examination was available. At autopsy, practically the entire right cerebellar hemisphere was replaced by a large hemorrhage which extended to the subependymal region of the fourth ventricle. None of the ventricles contained blood. The heart weighed 720 gm.

Case 7

E. H. (X-39-162), a colored female, 35 years old, had been treated for hypertension for at least 1 year. She was found dead by her daughter. Autopsy revealed massive hemorrhage into the right cerebellar hemisphere, with extension to all ventricles and basal cisternae.

Case 8

A white male, 65(?) years old, of unknown identity (X-39-178), was found dead. The cause of death was a large hemorrhage in the vermis of the cerebellum with more marked extension to the right lobe and a blood-clot cast of all ventricles.

Case 9

D. L. (X-40-95), a white male, 58 years old, was found dead. Examination of the brain showed excavation of one-half of the cerebellum by a large recent hemorrhage (Fig. 6). Blood-clot casts of the fourth and other ventricles, and limited subarachnoid hemorrhage, were present.

Case 10

M. deA. (X-41-117), a white female, 76 years old, died suddenly at home. At autopsy, the left lobe of the cerebellum was replaced by reddish blood clot with extension to the subarachnoid space.

Case 11

A white female, about 65 years of age, of unknown identity (X-39-137), was found dead in the street. Autopsy revealed an area of hemorrhage, 4 by 5 cm., involving the right dentate nucleus and regional white matter. The medial portion of the left lobe was also affected by the hemorrhage. Extension to the subarachnoid space was found.

Case 12

M. F. (X-41-270), a white female, 21 years old, in the sixth month of pregnancy, complained suddenly of severe dizziness while shopping. She had had no previous complaints referable to her pregnancy. Within a very short time after the onset of dizziness, she became comatose and died before medical attention reached her. At autopsy, a large recent hemorrhage, 8 by 6 cm., involved the vermis with limited extension to both adjacent hemispheres. Both dentate nuclei were preserved. Only moderate subarachnoid hemorrhage was noted locally. On section, a complete blood-clot cast of all ventricles was found. The heart weighed only 200 gm. No aneurysm or other unusual findings were found after a careful search.

Case 13

S. K. (A-38-99), a white female, 52 years old, known to have had hypertensive heart disease for many years, entered the hospital with diminished cardiac reserve and headache. The systolic blood pressure was 260 mm. and the diastolic pressure, 140 mm. The blood urea nitrogen was 56 mg. per cent. Because of drowsiness, uremic odor of the breath, papilledema, albuminuria and enlarged heart, a diagnosis of malignant nephrosclerosis was made. Autopsy confirmed the clinical impression. In addition, a small hemorrhage (2 cm. in diameter) was found in the left lobe of the cerebellum situated in the deep cortical layers near the junction of gray and white matter.

Case 14

J. K. (A-40-505), a white male, 52 years old, with a history of hypertension of 3 years' duration, entered with the complaint of inability to void. No obstructive genitourinary lesion was found to account for the retention. The blood pressure ranged from 200 to 280 mm., systolic, and 100 to 180 mm., diastolic. The blood urea nitrogen rose from 59 to 120 mg. per cent during 22 days in the hospital. No neurological symptoms or signs were noted. The immediate cause of death was an extensive bronchopneumonia of the left lower lobe. The kidneys showed advanced arteriolosclerosis, without necrotizing changes. The left ventricular myocardium was massively hypertrophied, the heart weighing 710 gm. The brain showed no unusual features, with the exception of the right cerebellar hemisphere, where an area of dark hemorrhage, 2 by 1 cm., was seen involving the cortex, with limited regional extension to the subcortical white matter (Fig. 7). The ventricles and subarachnoid space contained no blood.

Case 15

W. L. (A-41-284), a white male, 48 years old, entered with progressive urinary complaints, and died in uremia after 8 days, with signs of ascending urinary infection. No neurological symptoms or signs were noted during the course of his illness. The essential mechanism of death was an extensive cystitis and ureteropyelonephritis secondary to prostatic hypertrophy. A small hemorrhage, 2 mm. in diameter, was found in the right cerebellar hemisphere at the junction of the gray and white matter (Fig. 8). There was no extension to the subarachnoid space or ventricles.

Clinical Data

The two series will be compared in the following respects: age, sex, predominant symptoms, outstanding neurological signs and duration. The central nervous system lesions and the associated or related systemic pathology will be considered separately.

Age. In series A there were 8 cases in which the age was not stated and in series B, 2 such instances. The distribution of the remaining cases is presented in Table I.

Table I

Age Distribution by Decades of 114 Cases of Cerebellar Hemorrhage

Decade	Series A	Series B
1	4	1
2	11	0
3	13	1
4	6	1
5	10	1
6	14	4
7	23	3
8	18	2
9	2	0
	101	13

Sex. In series A, there were 61 males, 46 females and 2 cases in which the sex was not stated. In series B, 7 were males, and 8 females.

Symptoms. A complete and detailed account of the symptomatology of cerebellar hemorrhage may be culled from an analysis of the literature. Coma as a prominent symptom far overshadowed all other findings, having been recorded in 69 cases. In the order of frequency, the following additional symptoms were noted: vomiting (38), severe headaches (35), dizziness (16), ataxia (11), pain in the back of the neck (10), convulsions (5), recurrent mania (3), syncope (3), and blindness, abdominal pain and respiratory difficulty in 1 instance each.

The coma was usually of a profound type and as a rule premonitory aura were absent. Vomiting as the only symptom was rarely present and on a few occasions was described as projectile in nature. The distribution of the headache was not constant, but tended to be occipital rather than frontal in location. The dizziness and ataxia, when present,

did not differ from those noted in other forms of intracerebellar disease except for the dramatic suddenness of onset. In the 10 instances in which pain in the back of the neck was noted, a very typical history was that of a sensation of being "struck a blow on the back of the neck."

In 6 of the cases in series B, death was sudden, so that no definite history was available. No symptoms referable to the central nervous system were described in 4 other instances. In 3 of these, the hemorrhage was of such limited extent that the absence of symptoms is not surprising. It is of interest to note at this point that all 3 occurred in cases of uremia. In the fourth, although the hemorrhage ruptured into the fourth ventricle, the symptoms were masked by the advanced general debility of leukemia. Dizziness occurred in 4 of the remaining 5 cases and was characterized again by the dramatic suddenness of onset. Severe headache was noted in 1 case, while rapidly progressive coma, soon after the onset of symptoms, was observed in 2 instances.

Neurological Signs. There was no constant pattern of signs elicited in these cases of cerebellar hemorrhage. Of the 109 cases reviewed from the literature, by far the majority exhibited no neurological signs other than coma. In 18, hemiparesis or hemiplegia was noted and in two-thirds of these was on the side opposite the hemorrhage. Cranial nerve palsies were observed in 12 instances, the facial nerves in 6, the ocular nerves in 5, while the hypoglossal nerve was involved in the other. The facial palsy was homolateral in 4 of the 5 cases in which the site of the hemorrhage was stated. Conjugate deviation of the eyes was observed in 7 instances and was contralateral in 4 of the 5 cases in which the location of the lesion was described. Other less frequent and variable neurological signs included nystagmus (5), adiadokokinesis (2), opisthotonos (3) and positive Romberg's sign (2).

In series B, no significance can be attached to the relative incidence of symptoms and signs, inasmuch as 8 individuals died suddenly or before medical attention was available, and 3 represent incidental minimal lesions. One case showed marked nystagmus. Another revealed absence of the deep reflexes and unilateral flaccidity. In a third, admitted in coma, the deep reflexes were absent. In 13 cases, no neurological signs were present.

Duration. The duration of life from time of onset of symptoms until death may be seen from a study of Table II. That in series A there were only 3 examples of either sudden death or "found dead," while in the much smaller series B there were 6 such instances may be accounted for by the fact that many of the autopsies in the latter series were upon "sudden deaths" investigated by the Medical Examiner's Office. In

series A, 48 per cent of the patients died after 24 hours. None of those in series B lived for more than 9 hours.

Pathology in the Cerebellum

Location. In series A, hemorrhage occurred in the left lobe in 43 cases, in the right in 49 and in the vermis in 15. The corresponding figures for series B are 4, 8 and 3.

Table II
Duration of Life from Time of Onset of Symptoms until Death

	Series A		Series B	
Sudden death	3		6	
Less than 1 hour	9		3	
One to 12 hours	27		2	
Twelve to 24 hours	14		..	
Total (less than 24 hours)		53		11
One to 7 days	23		..	
One to 4 weeks	16		..	
One to 6 months	8		..	
More than 6 months	2		..	
Total (more than 24 hours)		49		0
Grand total		102		11

Size. The size of the hemorrhage was noted specifically in 104 of the 109 cases reviewed. In 84, the hemorrhage exceeded 5 cm. in diameter. In series B, 11 were larger and 4 smaller than 5 cm. Of the latter 4, 3 were incidental lesions, without clinical significance.

Extension of Hemorrhage. There were 7 examples of extension to the subarachnoid space in series A, and 4 in series B. Ventricular extension of the hemorrhage was more frequent and included 35 instances of this complication in series A. In 30 of these, the hemorrhage in the cerebellum was listed as larger than 5 cm. Of the 35 cases of intraventricular extension, 26 lived for less than 24 hours. In most of the cases cited in the literature, the findings in the ventricles were not mentioned specifically. Of the 8 cases of intraventricular extension in series B, 5 were found dead or died suddenly, 1 lived for 1 hour and 2 lived for 9 hours. Hemorrhage into the ventricles was definitely noted in 4 of the 6 cases of sudden death, while the condition of the ventricles was not specifically stated in the other 2 cases.

Associated Pathology

Cerebral Arteriosclerosis. Cerebral arteriosclerosis was mentioned in the protocols of 14 of the cases cited in series A. A quantitative estimation of the degree of sclerosis was not stressed. Cerebral arterio-

sclerosis of moderate to marked degree was noted in 3 of the 15 cases reported in series B.

Miscellaneous Cerebral Lesions. In series A, 1 instance each of gumma of the cerebellar vessels with rupture and hemorrhage, and of ruptured miliary aneurysms was noted.

Hypertrophy of the Heart. Cardiac enlargement was described in 11 cases in series A. As far as could be determined, no heart weights were recorded in these cases. In series B, 10 cases of hypertrophy of the heart were observed. In 1, the weight was not recorded, while in the others weight of the hearts varied from 420 to 720 gm., with an average of 565 gm.

Other Incidental Findings. In series A, 1 case each of hydatid cysts of the liver, healed rheumatic heart disease, cirrhosis of the liver and ulcerative colitis was mentioned. In series B, 3 cases of malignant nephrosclerosis and 2 cases of myelogenous leukemia were seen; 3 patients died in uremia. None of these 3 had focal symptoms of the central nervous system, and in all there were found only small hemorrhages, 2 mm., 2 cm., and 2 by 1 cm. in diameter, respectively. One patient expired during the sixth month of gestation and no associated pathological changes were found in any of the viscera. The heart in this instance weighed only 200 gm., and there was no demonstrable alteration of the cerebral vessels.

Comment

Determinations of the relative frequency of cerebellar as compared to cerebral hemorrhage have varied from 0.9 to 10 per cent.[2] In our own series, the ratio of cerebellar to cerebral hemorrhage was 15 per cent. As indicated in the discussion of the duration of symptoms, this higher incidence is explained by the inclusion of Medical Examiner's material. Of the 94 cases of spontaneous intracranial hemorrhage observed in the hospital, only 8, or 8.5 per cent, were of the cerebellar variety, whereas of the 21 instances of spontaneous intracranial hemorrhage that died outside of the hospital 7, or 33 per cent, were located in the cerebellum. Because this occurrence of cerebellar hemorrhage did not seem to be disproportionate to the relative mass of the cerebellum, the weights of cerebellum and cerebrum were recorded in 50 consecutive brains. The ratio between the average weights was 12.3 per cent. If no specific or peculiar factors were operative in the causation of cerebellar hemorrhage, one would expect this figure to represent the ratio of incidence of cerebellar to cerebral hemorrhage. Actually, the incidence in our series exceeds this figure. If the 3 cases of incidental minimal hemorrhage are excluded, it is of interest to note that the ratio

is reduced to 12 per cent, exactly the ratio of the weight of the cerebellum to the weight of the cerebrum. These findings contradict the general impression that cerebellar hemorrhage is a rare lesion, in contrast to cerebral hemorrhage. The alleged rarity previously stressed is explained in part by the failure to investigate adequately cases of sudden death, which constitute a considerable proportion of the present series.

Cerebellar hemorrhage tends to be fatal more rapidly than cerebral hemorrhage for several reasons. The proximity of vital medullary centers, the confining effect of the tentorium cerebelli and the relatively short tissue barrier from the subarachnoidal space and the ventricular system are all important factors. Extension of the hemorrhage into the ventricular system is a cause of sudden death in all forms of intracranial hemorrhage, and this complication occurs more often in cerebellar apoplexy. Of the 12 cases in which the cerebellar hemorrhage was the cause of death in our series, 8 showed intraventricular extension.

On the basis of simple anatomical and physiological grounds, there is no reason to expect that the factors responsible for cerebellar hemorrhage should differ in any major degree from those causing cerebral apoplexy. Actually, this is borne out by an analysis of associated pathological findings and case histories. Hypertensive cardiovascular disease was the most common associated finding. In only 2 of the cases was a heart of normal size recorded. This parallels the usual findings in cases of cerebral hemorrhage. Malignant nephrosclerosis, cerebral arteriosclerosis and myelogenous leukemia, common findings in association with cerebral hemorrhage, were noted in 8 instances. The identical nature of the contributory pathological factors in cerebellar and cerebral apoplexy has not been stressed in the literature heretofore.

The clinical syndrome of cerebellar hemorrhage is a variable one, in contrast to the typical example of cerebral hemorrhage. When the hemorrhage is small, the lesion is often silent, as might be expected. When the lesion is large, it is usually rapidly fatal. No characteristic syndrome, corresponding to that found in hemorrhage in the internal capsule and basal ganglia, is observed in cerebellar hemorrhage. The common site of cerebral hemorrhage is the internal capsule and regional tissue, a central location in the cerebrum. A similar central localization of cerebellar hemorrhage in the region of the dentate nucleus or vermis has been observed. In the three small incidental hemorrhages described in series B, however, these structures were not affected. In contrast, the small lesions were noted at the junction of the white matter and the cortex, usually at the periphery of the affected lobe. On the basis of

our own experience with these three cases of incidental hemorrhages, it is suggested that careful examination of the cerebellum be carried out in those dying of uremia.

Conclusions

1. Cerebellar hemorrhage is similar to cerebral hemorrhage in incidence and the contributory pathological factors are identical.

2. Cerebellar hemorrhage tends to be more rapidly fatal than cerebral hemorrhage and produces a more variable clinical syndrome.

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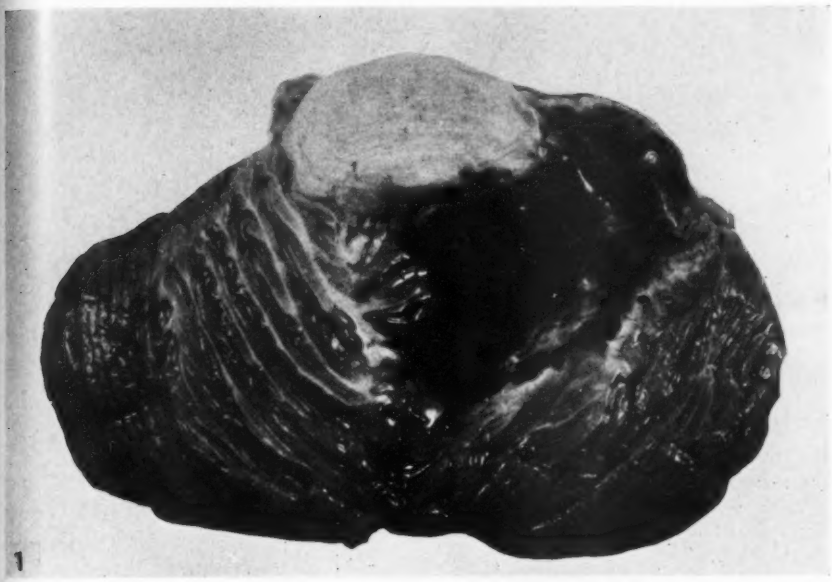
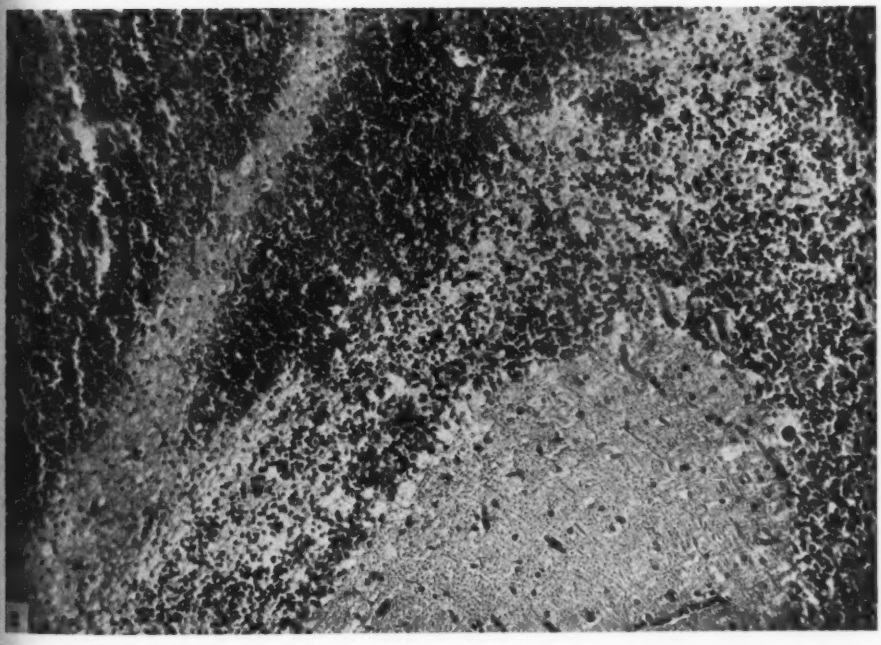
DESCRIPTION OF PLATES

PLATE 142

FIG. 1. Case 1. Massive hemorrhage of right lobe, extending to the vermis.

FIG. 2. Case 1. Low power photomicrograph of cerebellum showing leukemic infiltration and hemorrhage in white matter adjacent to molecular layer of cortex. × 80.





Mitchell and Angrist Spontaneous Cerebellar Hemorrhage

PLATE 143

FIG. 3. Case 1. High power photomicrograph of cerebellum showing leukemic infiltration at lower left and hemorrhage at upper left in white matter. × 205.

FIG. 4. Case 2. Hemorrhage into left lobe of cerebellum in region of dentate nucleus with extension to fourth and lateral ventricles.

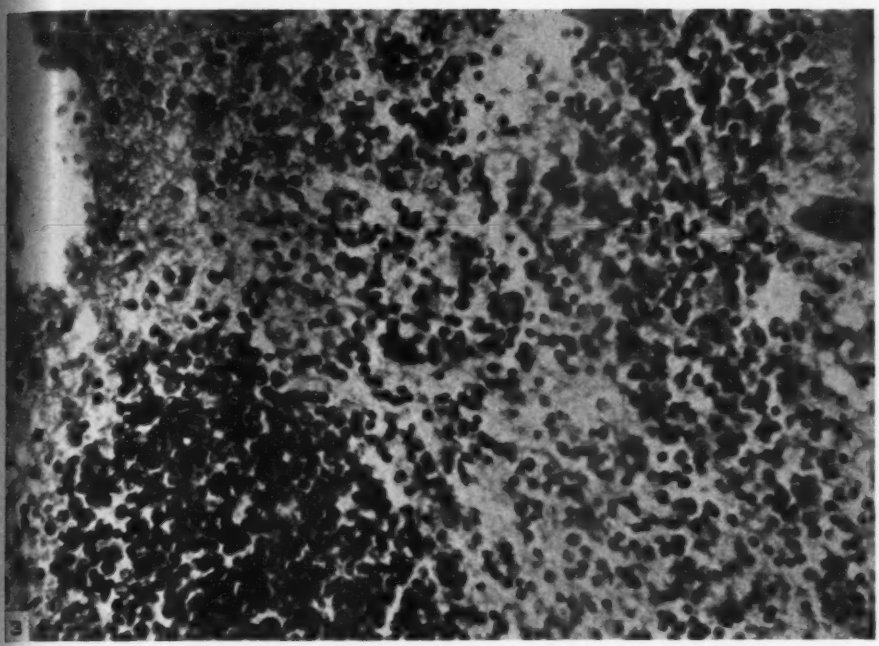


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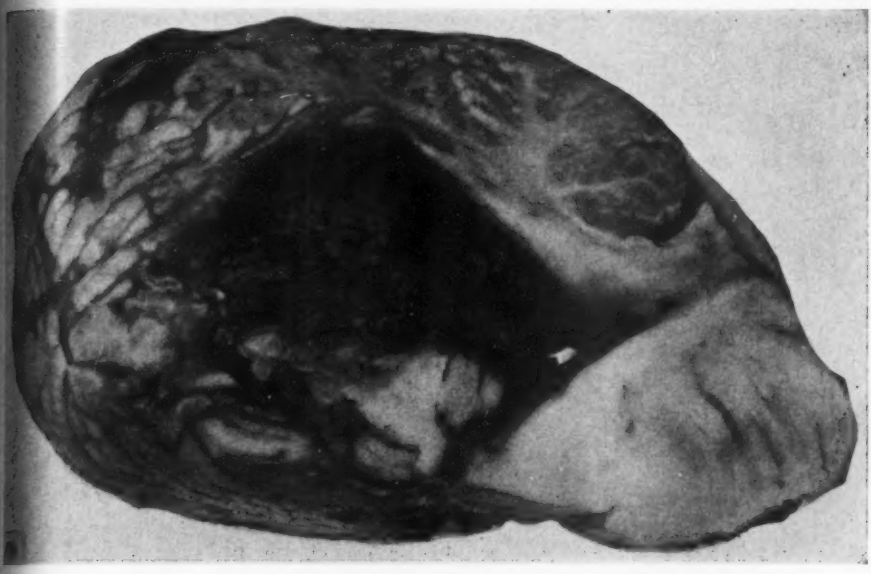
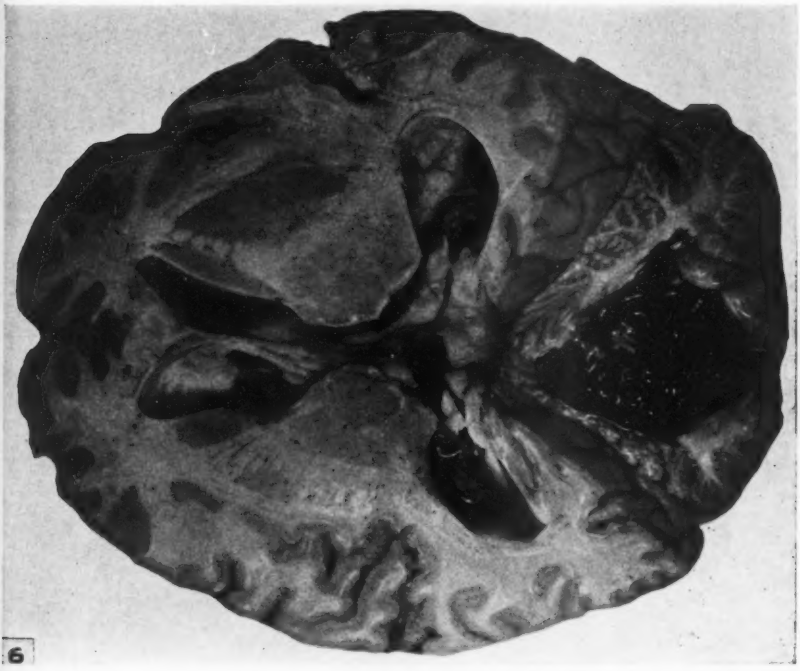
PLATE 144

FIG. 5. Case 4. Sagittal section of cerebellum, pons and upper medulla showing large hemorrhage in vermis extending to fourth ventricle.

FIG. 6. Case 9. Centrally placed massive hemorrhage in left lobe and vermis with extension to lateral ventricle.



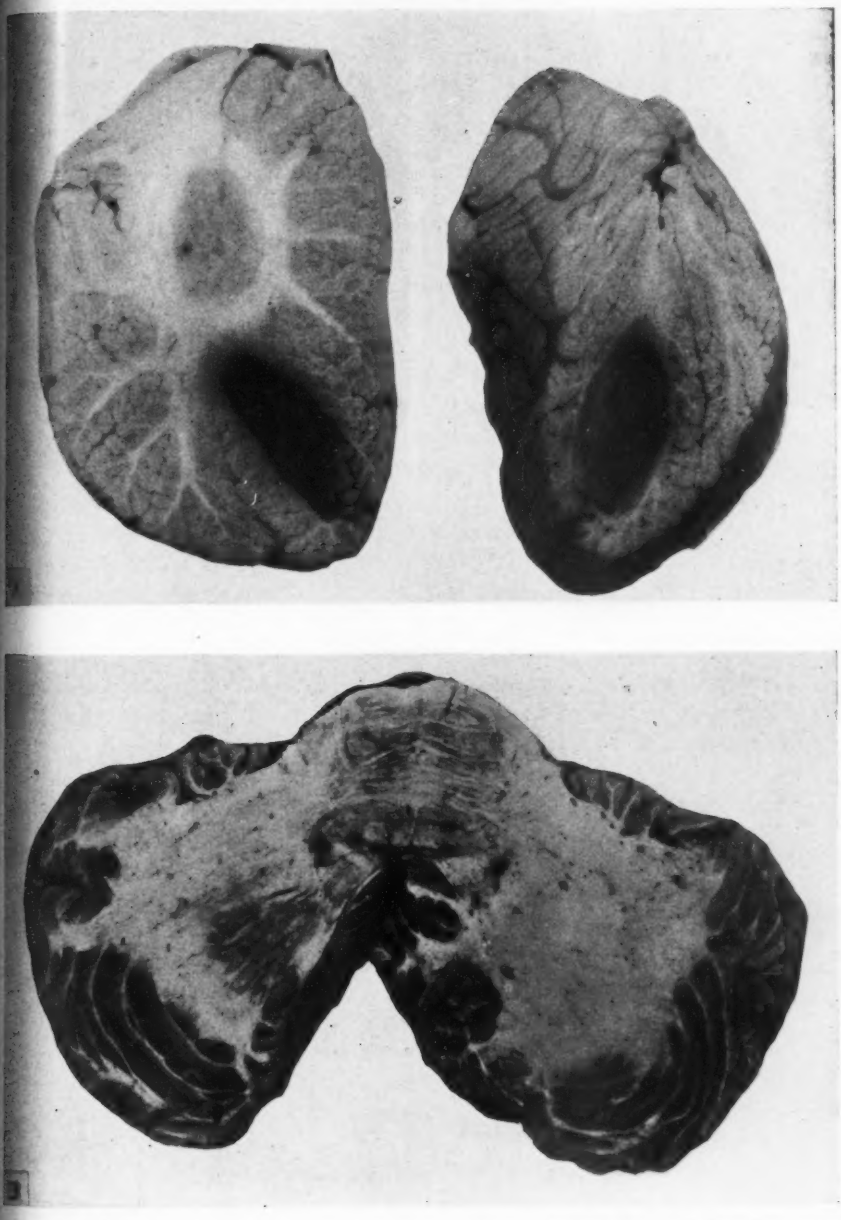


Mitchell and Angrist Spontaneous Cerebellar Hemorrhage

PLATE 145

FIG. 7. Case 14. Right lobe of cerebellum showing hemorrhage, 2 by 1 cm., in cortex and adjacent white matter.

FIG. 8. Case 15. Horizontal hemisection of cerebellum and pons through the region of the middle cerebellar peduncle, with an area of hemorrhage, 2 mm. in diameter, in right lobe at junction of gray and white matter.



Mitchell and Angrist Spontaneous Cerebellar Hemorrhage

ANLAGEN AND REST TUMORS OF THE LUNG INCLUSIVE OF "MIXED TUMORS" (WOMACK AND GRAHAM) *

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It is quite apparent from perusal of the literature relative to the classification of tumors of the lung that there is but little agreement upon this subject. The greatest attention is, of course, focused upon the study of tumors of bronchiogenic origin because of their relative importance and comparative frequency. The possibility of treatment by surgical methods and the intrinsic malignant or benign character of the neoplasm are factors of vital consideration to the patient. For a comprehensive dissertation upon these phases of the subject the reader is referred to the paper by Goldman and Stephens [1] with the accompanying discussions.

Aside from the representative and basic studies of Fried [2-4] upon bronchiogenic carcinomata, wherein aberrant growth arising from the basal cell of mature tissue structure is discussed, the most significant observation relating to the pleomorphism of pulmonary neoplasms in respect to both cytology and histological pattern is that of Womack and Graham [5] upon mixed tumors. We [6] have, in a previous communication, discussed the intricate embryological mechanism of the lung and the likelihood of anlagen and rest alterations as a source of neoplastic formation. The intimacy of the laryngotracheal ridge and esophageal development serves to explain the entodermal anomalies of epithelial structures as emphasized by Rector and Connerley.[7] The mesenchymal defects are exemplified in congenital cystic disease, which topic, in general, is well set forth by Sellors.[8] Conceding such formative digressions as one source of tumor origin, the possibility of bizarre and diverse histological manifestations becomes apparent. It is not unlikely that these factors have led to the confused deductions and to the various names objectively applied in efforts at classification.

The designation "mixed tumors" of the lung by Womack and Graham [5] has had the salutary effects of emphasizing the embryological course of certain pulmonary tumors and of carrying a reasonable implication that some of these are benign. Strictly speaking, many such tumors may be unidermal, but with the intention of both confirming and stressing the observations of Womack and Graham, it appears to us that the terms "anlagen" or "rest" tumors serve more fully to em-

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phasize and encompass their objective. Naturally, the benignancy or malignancy of such neoplasms are dependent upon the same histological criteria regardless of origin.

Material Studied

The first two tumors which suggested to us origin from anlagen or rests occurred in newborn, full-term infants who had survived 7 and 5 days respectively. Such distinctive intrauterine tumor growths immediately indicated embryological aberrations. The case histories and clinical findings are not important. There were evident respiratory difficulties and congenital atelectasis seemed probable.

CASE 1

In this infant surviving 7 days, it was found at necropsy that a tumor mass had replaced the entire lower lobe of the left lung. Upon sectioning, the mass was somewhat soft, and there were tubular structures, apparently dilated bronchi with thin walls, much like those noted in congenital cystic disease (Fig. 1). It is noteworthy that no irregular nodular formations were found except in that portion directed upward into the adjacent lobe. There had developed, for the greater part, a complete growth replacement of the normal structure of the lung. No other tumor masses and no metastasis were found.

Microscopical Examination

Sections prepared from various areas revealed, in general, the same histological picture. This consisted of irregularly distributed, tortuous branching tubules forming an apparently purposeless distribution of irregular alveolar spaces (Fig. 2). In many areas, infoldings or spur-like papillary invasions were noted. There was an occasional elongated tubular space having parallel sides and terminating in a simple bifurcation. These were suggestive of rudimentary bronchi. Tall, slender, columnar epithelial cells formed the linings for all the alveolar and tubular spaces. They revealed elongated, slender nuclei which were densely basophilic and presented a pale, slightly granular cytoplasm. In general these cells were arranged in a single layer, but occasionally they were stratified. The stromal structure was scant in amount and consisted of delicate, regularly arranged fibrous tissue. There was infrequently noted, in places where a larger amount of stroma was grouped, a more or less oval plaque of embryonal cartilage. Such plaques bore no direct relationship to the pseudobronchi and apparently were simple outgrowths of the mesenchymal development. Here and there in the stroma were found nutrient blood vessels.

CASE 2

This infant survived 5 days after full-term delivery. The upper lobe of the left lung presented two large, more or less rounded areas of ivory color which occupied the greater portion of its surface. Multiple radiating blood vessels, varying from approximately 1 mm. in diameter to minute dimensions, formed a cirsoid or telangiectatic arrangement over the surfaces (Fig. 3). Upon sectioning, these separate ivory-colored masses were found to merge beneath the surface. A structure of dense consistency was found to extend for a depth varying from approximately 1 mm. to 1 cm. It extended downward into a spongy reddish brown tissue which was less dense in character. The latter portion occupied approximately one-half of the upper lobe, the remaining tissue being crepitant and conforming to normal lung.

Microscopical Examination

In the pleural areas multiple blood-vascular sinuses were present within the connective tissue. These varied greatly in size and had thin walls. In many instances the lumen contained erythrocytes. Similar vessels appeared occasionally in the stromal projections within the underlying structure (Fig. 4). The nodules, as a whole, consisted of predominant young fibrous tissue. There were also numerous acinar spaces which were lined by low cuboidal epithelium and appeared in the aggregate as embryonal lung structure. Such, no doubt, had their origin from the entodermal layer of bronchial buds. A few irregularly shaped tubular spaces lined by columnar epithelium represented the more adult bronchiolar formations. It is probable that some of these latter spaces had certain continuity with the normally developed lung inasmuch as similar inflammatory cells were found scattered diffusely throughout spaces of both types.

CASE 3

A white male adult, 34 years of age, presented suggestive signs of an intrabronchial tumor, including hemoptysis. Specimens were obtained through bronchoscopic removal in 1935 and again in 1939. The interesting feature in this case is the dominance of one form of histological pattern in the primary biopsy specimen as compared to that removed 4 years later.

The specimen obtained in 1935 consisted of a small, rosette-shaped mass of tissue measuring approximately 6 by 5 by 4 mm. It was red, and soft. Upon removal, profuse bleeding occurred.

Microscopical Examinations

The sections presented a predominant mesenchymal derivative of cavernous hemangiomatous nature. There were numerous sinuses of irregular shape and size, lined with small rounded cells and containing numerous erythrocytes (Fig. 5). There were also similar spaces filled with cells resembling those present in embryonal bronchial glands.

The specimen removed in 1939 was an elongated oval portion of tissue of soft consistency and measuring 2 by 3 mm. in its greater diameters. Numerous more or less rounded acini were present. Many of these were lined with epithelium while others were completely filled with such cells. These cells presented comparatively large, deeply stained nuclei and only a small amount of light-staining protoplasm. They conformed to an embryonal or fetal gland type and were definitely of entodermal origin (Fig. 6). Only occasionally a sinus was seen containing blood, such as had been noted in the first specimen.

It seems evident that these two specimens were representative of mixed germinal layers. In the first a mesenchymal derivative was predominant while in the latter the tumor growth consisted to a great extent of elements of entodermal origin. This patient has just reported for observation. He is in excellent health and is free from all former signs and symptoms.

CASE 4

This individual was 19 years of age and had a history of occasional hemoptysis since the age of 7 years. Other clinical signs and symptoms were those usually present in a persistent intrabronchial polypoid growth wherein ball-valve action is present.

The specimen obtained through the bronchoscope was an irregularly shaped portion of tissue, of light pinkish red to pinkish gray color and somewhat firm in consistency. It measured approximately 7 by 5 mm.

Microscopical Examination

The sections revealed many more or less rounded acini with a lining of somewhat "ragged" epithelial cells with pale-staining nuclei and cytoplasm. These acini suggested a somewhat mature mucous gland structure; yet they were not of the fully developed adult type. In many of these spaces were found basophilic concretions of irregular shape, some of which resembled corpora amylacea (Fig. 7). While in portions the stromal structure showed nothing noteworthy, in other areas it formed very extensive plaques and presented extensive hyalinization (Fig. 8). In some of these hyalinized areas, cellular struc-

tures indicative of cartilage formation were seen. As has often been noted in growths in such locations, marked squamous-cell formation, metaplastic or aberrant, was present over the surface. The general characteristics of this tumor were indicative of mixed germinal origin inasmuch as both entodermal and mesenchymal structures appeared as participants in the neoplasm.

Summary

Four tumors of the lung, manifesting distinct evidences of origin either in anlagen or from more than one germinal layer, have been described. Two of these were of definite embryonal or anlagen origin, having developed during intrauterine life. In the first instance embryonal bronchial budding was perpetuated in a neoplastic mass. This was demonstrated by an aimless reduplication of tubular and immature alveolar structures indicative of a lack of orderly sequence of bud-branching. In the second case, evidence of both entodermal and mesodermal origin was revealed by areas of varied predominance of one or the other derivatives. In the third instance, less evidence of origin from mixed dermal layers was observed in each of two tissues removed for biopsy. Comparison, however, of the two specimens, between which there was an interval of 4 years, revealed a definite emphasis upon mesodermal structure in the first, while an entodermal character was clearly shown in the latter specimen. It is our opinion that the neoplasm in this second biopsy conformed closely in histological aspect to embryonal glands. The fourth example presented a neoplasm of mixed dermal components as revealed by the prevalence of elements of both layers.

It seems imperative, from the standpoint of the histological study of bronchiogenic tumors, that the embryological nature, as emphasized by Womack and Graham,[5] must receive serious consideration. In the study herein reported, bronchial anlagen origin is clearly established for certain of the tumors presented. Origin in an embryonal glandular rest is most probable in one instance.

While the biological characteristics of malignancy and benignancy are more or less constant regardless of the primary mature or embryonal origin of neoplasms, there are precedents for a benign nature for many tumors having the latter origin. Such serve to promote conservatism in the evaluation of the relative malignancy of the pulmonary growths which may belong to this group.

Note: We wish to express our appreciation to Dr. S. H. Colvin, through whose courtesy we obtained cases 3 and 4.

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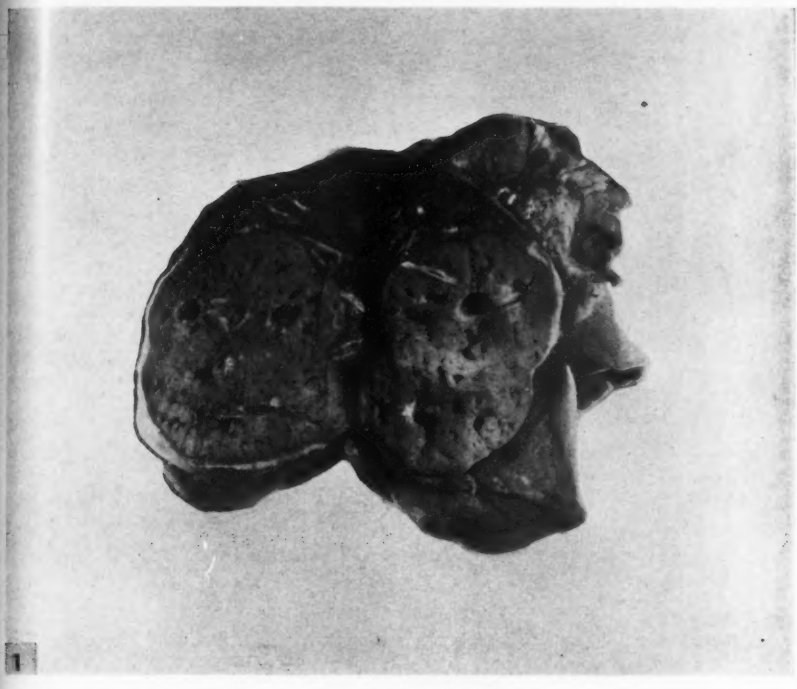
DESCRIPTION OF PLATES

PLATE 146

FIG. 1. Gross photograph of the left lung in case 1 showing a tumor mass replacing the entire lower lobe and compressing the upper lobe. The openings represent dilated tubules of apparently undeveloped bronchi analogous to those which occur in congenital cystic disease.

FIG. 2. Photomicrograph of the lung tumor in case 1 revealing branching tubules similar to those present in early embryonal lung structure. The lining consists of a single layer of columnar epithelium. × 300.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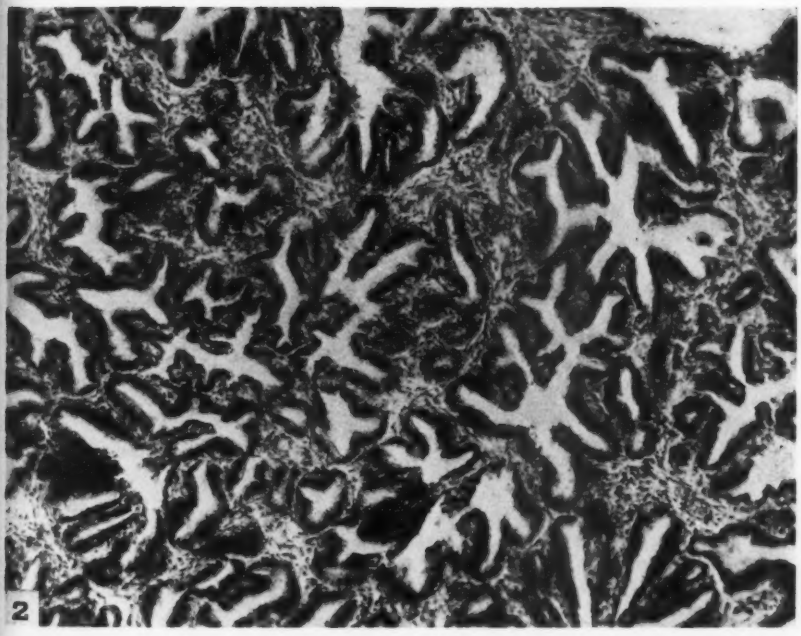
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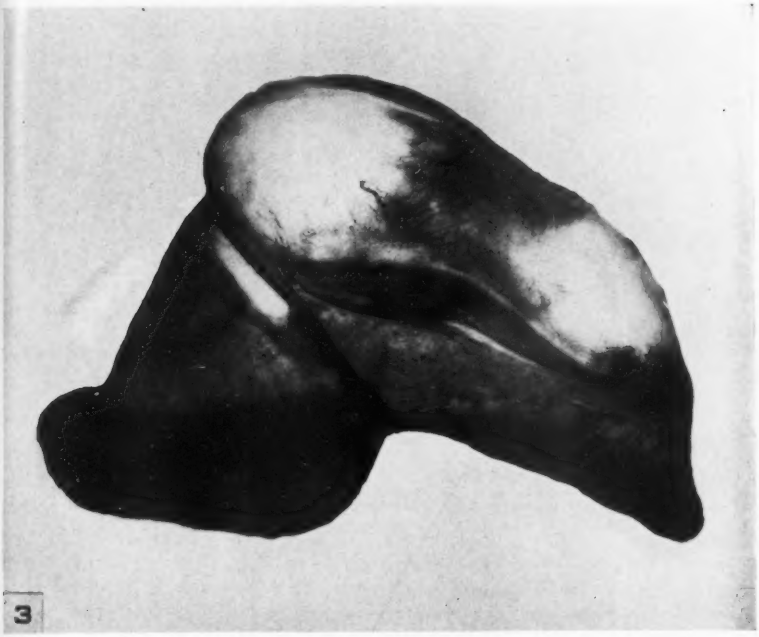
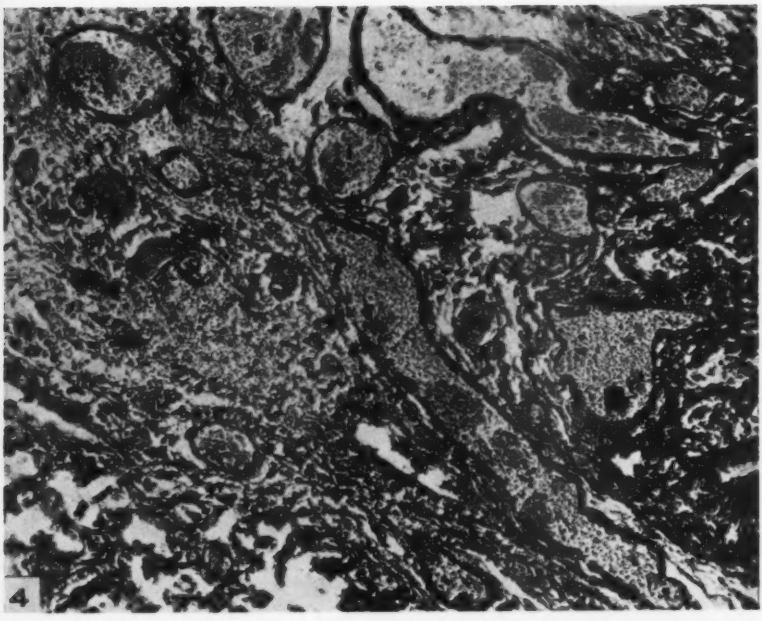
PLATE 147

FIG. 3. Gross photograph of the right lung of case 2. Large tumor masses are seen in the upper lobe of the right lung. Dilated vascular ramifications are present.

FIG. 4. Photomicrograph of a section of the tumor in case 2 wherein cavernous sinus formation and rudimentary alveolar arrangements of lung structure can be noted. × 200.



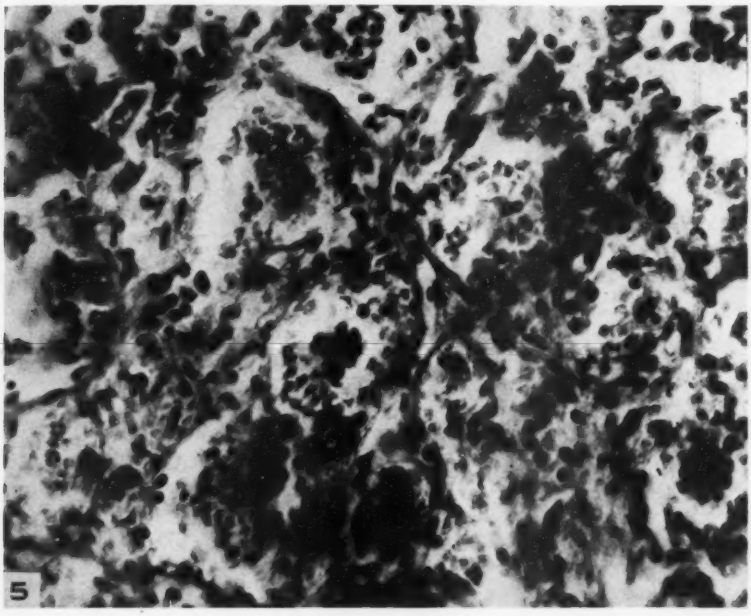


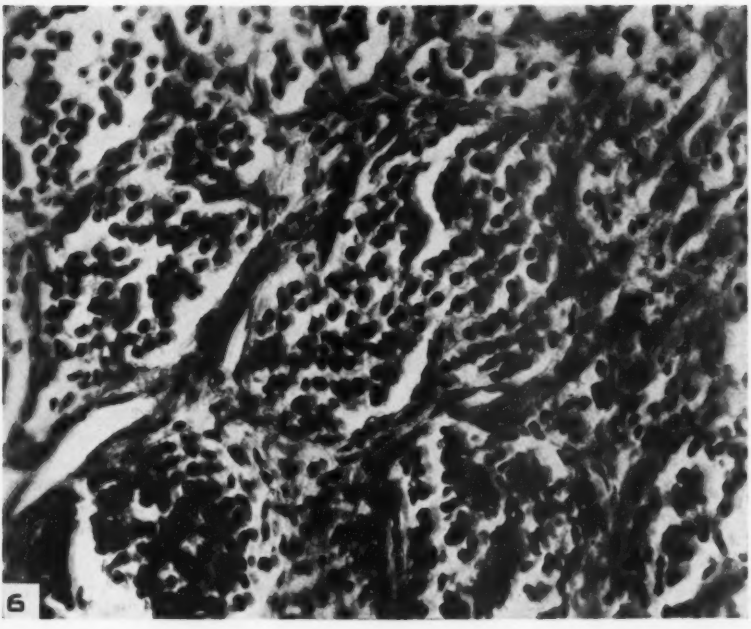
Harris and Schattenberg Anlagen and Rest Tumors of the Lung

PLATE 148

FIG. 5. Photomicrograph of the biopsy specimen in case 3, obtained in 1935, in which angiomatous formation is predominant. Numerous vascular spaces containing erythrocytes are shown. × 500.

FIG. 6. Photomicrograph of the biopsy specimen of case 3, removed 4 years later (1939) in which embryonal glandular structure prevails. Numerous acini contain round and polyhedral cells with deep-staining nuclei and pale protoplasm. × 500.



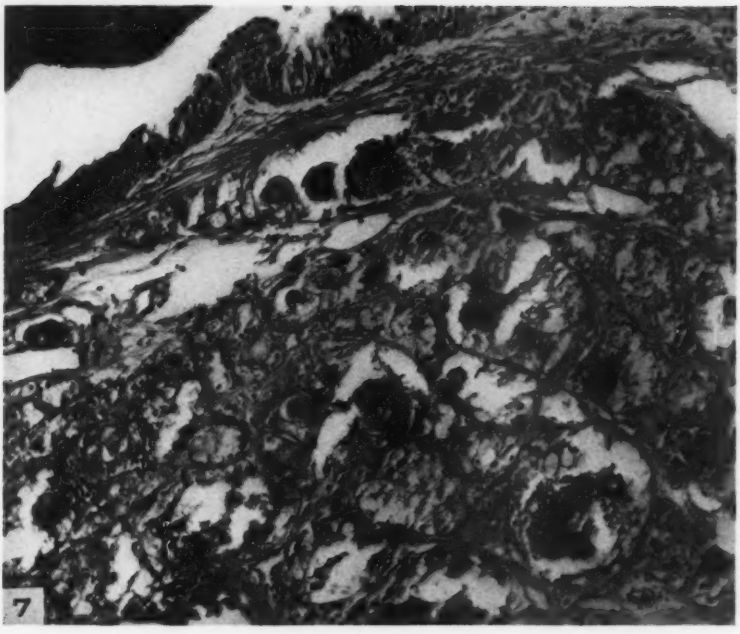


Harris and Schattenberg Anlagen and Rest Tumors of the Lung

PLATE 149

FIG. 7. Photomicrograph of the biopsy specimen of case 4, revealing glandular acini, many of which contain concretions. The lining cells are pale and irregular in outline. The columnar epithelium of the bronchial lining above shows squamous cell metaplasia in the upper left portion of the field. × 250.

FIG. 8. Photomicrograph of the biopsy specimen of case 4, showing an area wherein extensive chondromatous structure prevailed. A few scattered glandular acini are present. × 250.



Harris and Schattenberg Anlagen and Rest Tumors of the Lung